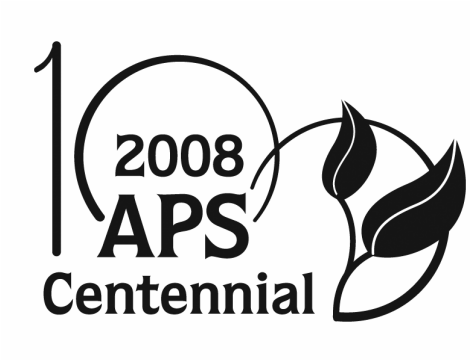
Blast Interfacial Complex, a novel in planta structure that accumulates effector proteins of rice blast fungus Magnaporthe oryzae

C. Khang, Romain Berruyer, S. Park, P. Kankanala,, K. Czymmek, S. Kang, B. Valent



2008 APS Centennial Meeting
Abstracts of Presentations

Abstracts submitted for presentation at the APS 2008 Centennial Meeting in Minneapolis, Minnesota, July 26–30, 2008 (including abstracts submitted for presentation at the 2008 APS North Central Division Meeting). The abstracts are arranged alphabetically by the first author's name.

Detection of an unknown virus in potato seedlings grown from true seed introduced from South America

J. A. ABAD (1), C. Loschinkohl (1), M. Smither (1)
(1) USDA APHIS PPQ PHP PSPI PGQP, Beltsville, MD, USA
Phytopathology 98:S9

While testing seedlings from 30 true potato seed accessions from South America using biological and molecular tests, we did not detect a pathogen in accession JCM-23, even though some seedlings showed severe upper leaf deformation and necrosis. Tests included mechanical inoculations onto 14 indicator plants, ELISA, and RT-PCR. A pathogen from JCM-23 was not mechanically transmitted in several attempts, yet it infected healthy potato plants via grafting. Tubers harvested from infected plants did not show any symptoms. However, plants grown from two such tubers showed strong necrosis, leaf deformation and rugosity ('frog' skin) as secondary symptoms. Double stranded RNA (dsRNA) analysis uncovered two species; a top 4 kb and a bottom 1.3 kb fragment. Cloning from dsRNA and sequencing are in progress. A preliminary result of sequence analysis shows that accession JCM-23 has a virus. This may be a potentially dangerous seed-transmissible virus affecting potatoes which was intercepted using a newly incorporated grafting method. This method not only improved the efficiency of the quarantine testing in our program, but also prevented the introduction of a putative new potato pathogen into the USA.

Aflatoxins and fumonisins enhanced by corn infected with common smut

H. K. ABBAS (1), R. M. Zablotowicz (3), C. A. Abel (2), H. Bruns (1)
(1) USDA-ARS, CG&PRU, Stoneville, MS 38776, USA; (2) USDA-ARS, SIMU, Stoneville, MS 38776, USA; (3) USDA-ARS, SWSRU, Stoneville, MS 38776, USA
Phytopathology 98:S9

Mycotoxins produced by Aspergillus and Fusarium species when present in corn, can cause serious toxicological problems in animals and humans. In addition, corn can also be infected with Ustilago maydis, the causal agent of common smut, which produces structures that are highly valued as edible food in certain cultures. Little is known about the relationship between mycotoxin contamination in corn infected with common smut. Field studies conducted in 2006 and 2007 in Mississippi evaluated the incidence of common smut, aflatoxin and fumonisin levels in near isogenic Bt (Pioneer brand 34B24Bt, Cry1Ab event MON810) and non-Bt (Pioneer brand 34B23) hybrids. In 2006, a high degree of common smut was observed (~ 17% infected ears) with lower levels in 2007 (~3%). There was no significant effect of the Bt gene. At harvest, grain from smut infected corn contained 2437 μg kg^{-1} total aflatoxin compared to ~ 50 μg kg^{-1} in corn not infected with smut in 2006 with no effect of hybrid. In 2007, smut infected non-Bt corn had aflatoxin levels of 1898 μg kg^{-1}, and corn Bt infected had levels of 183 μg kg^{-1} total aflatoxin, while <30 μg kg^{-1} total aflatoxin was found in smut-free corn from both hybrids. Fumonisin levels were five to ten-fold higher in smut infected corn in both years with no significant effect of the Bt trait. These studies show a high incidence of mycotoxins associated with smut infected corn. Further studies on mycotoxin contamination should be conducted to develop techniques to control toxin contamination in smut to be used as an edible food product.

Detection of high concentrations of organic acids in fish emulsion and their role in pathogen or disease suppression

P. A. ABBASI (1), G. Lazarovits (1), B. Weselowski (1)
(1) Agriculture and Agri-Food Canada, London, Ontario, Canada
Phytopathology 98:S9

Fish emulsion (FE) is a nutrient-rich organic by-product of fish industry mainly used as a fertilizer, but its disease suppressing effect against foliar and soilborne diseases has been demonstrated as well. FE amendment to a sandy loam soil reduced the viability of *Verticillium dahliae* microsclerotia in a dose response manner with a reduction of 39 and 74% after day 1, 87 and 98.5% after day 3, and 95 and 99% after day 6 with 1 and 2% FE, respectively. The immediate kill of microsclerotia indicated that FE may contain toxic substances. Analysis of FE revealed high quantities (400 mmol/L) of volatile and non-volatile organic acids in FE and some of these are known toxicants. Glycolic, acetic, formic, n-butyric, and propionic acids were the major organic acids detected in FE at the proportions of 52.5, 26.9, 7.9, 7.2, and 4.7%, respectively. In solution assays, the viability of *V. dahliae* microsclerotia treated for 24 h in 2, 5, and 10% FE (pH 3.6-3.0) or a mixture of organic acids (pH 4.1-3.9) equivalent to the proportions in FE was reduced by 94 and 91%, 97 and 98%, or 99%, respectively over the control. The viability of microsclerotia was increased when the treatment solutions were buffered to pH 6.0. This study suggests that presence of these toxic organic acids in FE may have a role in pathogen or disease suppression by FE.

Tolerance to *Cucurbit yellow stunting disorder virus* in cucumber is not correlated with a delay in virus movement

Y. ABOU JAWDAH (1), S. Eid (1), H. Atamian (1), M. Havey (2)
(1) American University of Beirut, Beirut, Lebanon; (2) University of Wisconsin, Madison, USA
Phytopathology 98:S9

Cucurbit yellow stunting disorder virus (CYSDV) is an emerging crinivirus causing significant yield losses to cucurbit crops. Previous studies showed that of over 120 cucumber accessions evaluated for resistance against CYSDV, no immune or highly resistant accessions were detected, but a limited number of accessions were tolerant and showed delayed expression of symptoms, milder final symptoms and lower percentages of infected plants compared to susceptible varieties. This study evaluated the relative sensitivity of CYSDV serological detection methods as compared to nucleic acid based methods and reports that in a susceptible germplasm, CYSDV was detected 5 days post

inoculation (DPI) by RT-PCR or by tissue print immunoassay (TPIA), and 8-9 DPI by dot blot immunoassay (DBIA) or ELISA. Time course studies at 3, 5, 8 and 14 DPI, using TPIA, revealed that tolerance to CYSDV in three tolerant cucumber germplasms was not correlated with restricted or delayed virus movement.

Isolation of chitinase gene induced during infection of *Vicia faba* by *Botrytis fabae*

N. M. ABOU-ZEID (1)
(1) Plant Pathology Research Institute, ARC, Giza 12619, Egypt
Phytopathology 98:S10

The moderately resistant (Giza 716) and the susceptible (Giza 429) faba bean cultivars were used to identify some pathogenesis related proteins (PRs) associated with infection by chocolate spot disease. One isolate of *Botrytis fabae* purified from a plant sample taken from Nubaria location (Behera governorate, Egypt) was used in the artificial infection experiment. Qualitative and quantitative analyses were carried out on all protein banding patterns of the healthy and the infected faba bean leaves harvested at 8, 24 and 48 hr after inoculation. Data revealed that a 26 kDa protein band was more intensive 8, 24 and 48 hr after inoculation in cultivar Giza 716. In addition, a 29 kDa protein band appeared after 24 and 48 hr. Furthermore, in cultivar Giza 429, 54 kDa protein band appeared after 8, 24 and 48 hr post inoculation and 28 and 20 kDa appeared after 24 hr post inoculation. Reverse-Transcription (RT-PCR) showed that chitinase gene is expressed at very early stages in infected faba bean leaves. DNA fragment at molecular weight 900 bp appeared at 8, 24 and 48 hr after inoculation and disappeared in the healthy plants. The amplified products were cloned into pGEM-T Easy vector. Four clones named (PNAM1, PNAM2, PNAM3 and PNAM4) were selected for validation. The recombinant plasmids PNAM1, PNAM2 were verified for the presence of the Chitinase gene coding sequences by using both specific and universal primers in PCR. BNAM1-Chit-EG gene sequence showed 58.15% similarity when aligned with other Chitinase genes published in the gene bank.

Evaluation of fungicides for control of Phytophthora blight of watermelon in North Carolina and South Carolina

M. L. ADAMS (1), G. Holmes (1), S. Kousik (2)
(1) NC State University, Raleigh, NC, USA; (2) USDA-ARS, Charleston, SC, USA
Phytopathology 98:S10

Phytophthora blight, caused by *Phytophthora capsici*, is an important disease of cucurbits in the eastern U.S. Fungicides, crop rotation, and water management are recommended to control the disease. Watermelon (*Citrullus lanatus*) poses a particularly difficult challenge to disease control because of the duration of fruit-soil contact. In each state, fungicides were used at labeled rates and applied at approx. 7-day intervals. Disease incidence was recorded (No. fruit rotted by *P. capsici*/ total number of fruit). Disease incidence in non-treated plots averaged 63% across 2 trials. In SC in 2006, Captan provided 68% disease control (calculated as % of non-treated) while mandipropamid (Revus) controlled disease at 66%. When Revus was combined with phosphonate (ProPhyt) and fixed copper (Kocide 2000) in SC, disease control was 74%. In 2007 in NC, captan, mandipropamid and folpet (Folpan) controlled disease at 69%, 66%, and 65%, respectively, but when captan was combined with Kocide, disease was controlled at 85%. In 2006 in SC, the same treatment of captan + Kocide provided only 45% disease control and in 2007 in NC, Revus + ProPhyt + Kocide controlled disease at only 48%. These results show that product performance varies by year and location and even the best treatment may not provide commercially acceptable levels of control in watermelon.

Differential gene expression in wheat in response to Ptr ToxA produced by *Pyrenophora tritici-repentis*

T. B. ADHIKARI (2), J. Bai (1), M. Myrfield (2), S. Ali (2), N. C. Gudmestad (2), J. B. Rasmussen (2)
(1) Kansas State University, Manhattan, KS 66506; (2) North Dakota State University, Fargo, ND 58105
Phytopathology 98:S10

Tan spot, caused by *Pyrenophora tritici-repentis*, is one of the most destructive foliar diseases of wheat. The fungus produces Ptr ToxA, a toxin that causes the development of necrosis in sensitive wheat. The molecular mechanisms underlying host reaction to Ptr ToxA are poorly understood. Wheat GeneChip® Genome Array was used to identify differentially expressed genes in sensitive and insensitive wheat mutant lines. Gene expression was assessed 0, 4, 12, 24, and 48 hrs after Ptr ToxA infiltration. Data analysis revealved maximum differential expression (>2 fold) was observed at 48 hrs where 1015 genes were down-regulated and 48 genes were up-regulated. In particular, signal transduction genes (e.g., receptor kinase-like protein, serine threonine-protein kinase) and defense-related genes (e.g., beta-1, 3-glucanase, chitinase, peroxidase, lipoxygenase) known to involve in basal defense were down-regulated at 48 hrs. A total of 124 genes were highly expressed across time-points, and 20 genes belonging to several functional categories were selected and validated by real-time PCR. The function of selected genes will be investigated by virus induced gene silencing (VIGS), and gene-specific primers will be evaluated for marker-assisted selection in wheat breeding programs.

The global genetic structure of *Pyrenophora tritici-repentis* populations

T. B. ADHIKARI (1), S. Ali (1), M. Myrfield (1), R. R. Burlakoti (1)
(1) North Dakota State University, Fargo, ND 58105
Phytopathology 98:S10

Pathological and molecular analyses were performed to determine the population structure of *Pyrenophora tritici-repentis* from several wheat-producing countries in Asia, Australia, Europe, North America and South America. Strains collected from each continent were regarded as a "population". Over 400 strains of *P. tritici-repentis* representing from five populations were inoculated on wheat differential cultivars. The genetic structure of all populations examined were determined using PCR amplified sequences from genes encoding two toxins (Ptr ToxA and Ptr ToxB) produced by the fungus and by 12 microsatellite markers. The North American population consisted of races 1 to 5, while the Australian population had only race 1. The European population contained races 1, 2, and 4. The Asian and South American populations contained both races 1 and 2. The *ToxA* gene was detected in most populations. The genetic diversity was high for all populations, as evidenced by the presence of similar frequencies of common microsatellite alleles. The genetic differentiation, gene flow and linkage disequilibrium between populations will be presented.

Identification of plant reservoirs and genome characterization of Squash vein yellowing virus, causal agent of viral watermelon vine decline in Florida

S. ADKINS (2), W. Li (5), M. E. Hilf (2), W. W. Turechek (2), C. S. Kousik (3), C. A. Baker (1), S. E. Webb (4)
(1) Florida Department of Agriculture and Consume Services, Division of Plant Industry, Gainesville, FL, USA; (2) USDA-ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL, USA; (3) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC, USA; (4) University of Florida, Department of Entomology and Nematology, Gainesville, FL, USA; (5) University of Florida, Lake Alfred, FL, USA
Phytopathology 98:S10

Squash vein yellowing virus (SqVYV) was identified in cucurbits in Florida in 2005 and shown to be whitefly-transmissible and to induce a previously observed watermelon vine decline and fruit rind necrosis. Only cucurbits have been determined to be hosts for SqVYV so common cucurbit weeds in south Florida were examined as potential reservoirs of SqVYV. Over 40% of 86 balsam-apple (*Momordica charantia*) plants collected from watermelon growing areas with previously reported cases of vine decline were found to be infected with SqVYV. Creeping cucumber (*Melothria pendula*) was determined to be an experimental host for SqVYV. Collectively, these results demonstrate that cucurbit weeds can provide reservoirs for SqVYV in Florida. Sequencing of the SqVYV genomic RNA showed one large open reading frame encoding a single polyprotein, a typical genome organization for most members of the family *Potyviridae*. Phylogenetic analysis of the ten mature proteins predicted to be derived from the SqVYV polyprotein supports classification of SqVYV as a novel species within the genus *Ipomovirus*. The presence of P1a and P1b proteins, and the absence of an HC-Pro protein, makes the genome organization of SqVYV similar to *Cucumber vein yellowing virus* but different from *Sweet potato mild mottle virus*, both recognized members of the genus *Ipomovirus*. This indicates that the taxonomy of the genus *Ipomovirus* needs to be re-examined and a new genus created within the family *Potyviridae* to accommodate the observed discrepancies in ipomovirus genome organization.

***Phytophthora nicotianae* zoospores evade pressure and agitation stress but are completely destroyed by CO_2 injection**

M. O. AHONSI (2), T. J. Banko (2), S. R. Doane (1), A. O. Demuren (1), W. E. Copes (3), C. X. Hong (2)
(1) Department of Mechanical Engineering, Old Dominion University, Norfolk, VA 23529; (2) Hampton Roads Agricultural Research and Extension Center, Virginia Tech, 1444 Diamond Springs Road, Virginia Beach, VA 23455; (3) USDA-ARS Southern Horticultural Laboratory, 810 Hwy 26 West, Poplarville, MS 39470
Phytopathology 98:S10

Phytophthora nicotianae is a known pathogen of numerous herbaceous and some woody ornamental plants, and is commonly isolated from recycled irrigation ponds. Zoospores are the most important propagules of *Phytophthora* spp. Using simulated recycled irrigation water we investigated

the survival of *P. nicotianae* zoospores as affected by hydrostatic pressure, agitation, and aeration with CO_2 or air. Exposing zoospores to hydrostatic pressure of 840 kPa for 8 min or agitation of mixing intensity G = 6483 1/s for 4 min did not kill any zoospores. However, bubbling CO_2 into zoospore-infested water at 110.4 ml (0.2 g)/min for 5 min consistently killed up to 81% of the zoospores. Further extending CO_2 injection up to 30 min did not increase percent zoospores killed although fewer were killed with a shortened injection time. When we exposed zoospores to CO_2 pressure of 630 kPa (16.3 g CO_2) or 70 kPa (3.85 g CO_2) for 30 seconds or longer, percent zoospore kill did not differ from one another and did not differ from bubbling CO_2 at 110.4 ml/min for 5 min. In contrast, when the same treatments were done using pressurized air in place of CO_2, all zoospores survived. In further experiments, when we minimized cyst formation during zoospore-infested water preparation by avoiding vigorous shaking, CO_2 injection consistently resulted in over 98% zoospore kill. We concluded that the percent zoospores not killed by CO_2 injection in previous experiments were zoospores that had encysted before exposure to CO_2. Similarly, hydrostatic pressure and agitation treatments induced cyst formation and consequently allowed 100% survival. Results indicate that CO_2 treatment may be a promising alternative technology for disinfesting recycled irrigation water contaminated with *P. nicotianae*.

Root rot fungi succession during Cassava (*Manihot esculenta*. Crantz) tuberous root development in different ecological zones of Nigeria

S. O. AIGBE (1), S. U. Remison (1), R. Bandyopadhyay (2)
(1) Department of Crop Science, Ambrose Alli University, PMB 14 Ekpoma, Nigeria; (2) IITA, PMB 5320, Ibadan, Nigeria
Phytopathology 98:S11

Root rot fungi succession during cassava tuberous root development was investigated in Ibadan (Derived savanna), Sabongidda-Ora and Onne (Humid forests) of Nigeria. Two improved cassava varieties (TMS 30572 and TMS 4 (2) 1425) and one local (TME-1) were planted in a randomized complete block design with 3 replications. Isolation of associated fungi was done from tuberous and fibrous root samples collected at 3, 6, 9 and 12 months after planting (MAP). Six species (*Fusarium solani*, *Fusarium oxysporum*, *Botryodiplodia theobromae*, *Trichoderma harzianum*, *Rhizopus stolonifer*, *Aspergillus niger*) and one unknown fungus were isolated frequently from growing roots. A clear relationship was demonstrated between growth stage, location, fungal succession and root rot incidence/severity. During the two planting seasons of this study and also throughout all locations, fibrous roots generally supported more diversity and higher frequencies of fungal growth than the tuberous roots. *Fusarium oxysporum* was usually an early to mid stage colonizer of root tissue while *B. theobromae* was a late colonizer. *Botryodiplodia theobromae* and *F. solani* were normally absent or rare during dry season periods at 6 MAP but re-emerged when rainy season resumed at 9 MAP till 12 MAP. *Fusarium oxysporum* dominated during the dry season period at 6 MAP. In the trial, local TME-1 genotype was the most colonized while improved TMS 4(2)1425 genotype was least colonized. In Ibadan and Sabongidda-Ora during the first planting season, *F. solani* was the most prevalent colonizer while *B. theobromae* was the most prevalent in Onne.

Multiplex PCR method for the detection of *African cassava mosaic virus* and *East African cassava mosaic Cameroon virus* in cassava

O. J. ALABI (1), L. P. Kumar (2), R. A. Naidu (1)
(1) Department of Plant Pathology, Washington State University, Irrigated Agric. Research and Extn. Center, Prosser, WA 99350; (2) International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria
Phytopathology 98:S11

Cassava mosaic disease (CMD) is a significant concern to cassava production in sub-Saharan Africa. Among the six whitefly-transmitted geminiviruses (family *Geminiviridae*, genus *Begomovirus*) infecting cassava, *African cassava mosaic virus* (ACMV) and *East African cassava mosaic Cameroon virus* (EACMCV) are prevalent in Nigeria. In this study, we have developed a multiplex PCR method for the simultaneous detection of ACMV and EACMCV in CMD-infected cassava. The protocol uses a set of primers consisting of an upstream primer common for both viruses and two down stream primers specific for each virus to distinguish the amplified DNA fragments. One set of primer combinations specific to the coat protein (CP) amplified 540 base pair (bp) and 655 bp fragments for EACMCV and ACMV, respectively. Another set of primer combinations specific to the replicase (Rep) amplified 368 and 650 bp fragment for ACMV and EACMCV, respectively. In both assays, primers that can amplify a 181 bp fragment of the large subunit of ribulose bisphosphate carboxylase oxygenase L (RubiscoL) were included as an internal control to determine the reliability of multiplex PCR method. The results showed that Rep-specific primers are more suitable than CP-specific primers for the simultaneous detection of ACMV and EACMCV in PCR assays. A simplified sample preparation method, in place of conventional DNA extraction, was adapted for use in multiplex PCR detection of ACMV and EACMCV. The multiplex PCR method was validated for the detection of ACMV and EACMCV in CMD-infected cassava samples obtained from farmers' fields in Nigeria. The method can be used for the routine detection of cassava mosaic begomoviruses in many African countries.

Distribution of *Diplodia pinea* and *D. scrobiculata* in red and jack pine forests of Minnesota

J. S. ALBERS (2), D. R. Smith (1), G. R. Stanosz (1)
(1) Department of Plant Pathology, University of Wisconsin-Madison, WI 53706, USA; (2) Division of Forestry, Minnesota Department of Natural Resources, Grand Rapids, MN 55744, USA
Phytopathology 98:S11

Diplodia pinea (syn. *S. sapinea* A group) and *D. scrobiculata* (syn. *S. sapinea* B group) cause shoot blight and canker diseases of red pine (*Pinus resinosa*) and jack pine (*P. banksiana*) in the northcentral and northeastern USA. Their distribution in Minnesota was studied by examination of seed cones (on which these fungi sporulate). 100 cones collected from the forest floor of each of 109 red pine stands and 28 jack pine stands were visually examined for *Diplodia* pycnidia and conidia. At least one of these fungi was detected from 106 of 109 red pine stands and from all jack pine stands. Mean frequencies of positive red and jack pine cones, respectively, were 27% (range 0–84%) and 12% (range 2–41%). PCR assays confirmed pathogen identity for subsets of cones. *D. pinea* was detected from cones collected at 102 of 109 red pine stands (69% of red pine cones tested), and 18 of 28 jack pine stands (18% of jack pine cones tested). In contrast, *D. scrobiculata* was detected from cones collected at only 26 of 109 red pine stands (7% of red pine cones tested), but 26 of 28 jack pine stands (79% of jack pine cones tested). These fungi sometimes co-occurred in stands of either host, and occasionally both were detected from individual cones of either host. Although differences between *D. pinea* and *D. scrobiculata* in host association, presence at a given location, and frequency of occurrence at a given location were apparent, each was found across the entire area surveyed.

Pathogenic variation in *Pyrenophora tritici-repentis* from Arkansas and evaluation of wheat genotypes for resistance to multiple races of *P. tritici-repentis*

S. ALI (1), E. Milus (2), T. B. Adhikari (1)
(1) North Dakota State University, Fargo, ND 58105; (2) University of Arkansas, Fayetteville, AR 72701
Phytopathology 98:S11

Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is an important foliar disease of wheat worldwide. Multiple races of Ptr have been identified. Planting resistant cultivars appear to be the most effective strategy in managing tan spot in wheat. In this study, pathogenic variation of 93 Ptr isolates collected from Arkansas was evaluated on wheat differential cultivars. Forty-two of the 93 isolates were analyzed using PCR-based primer set specific to the *ToxA* gene. Twenty wheat genotypes from Arkansas were evaluated for their reaction to races 1, 3, 5 and one unclassified isolate Ark Lon B2 at seedling stage under greenhouse conditions. The genotypes also were evaluated for their sensitivity to Ptr ToxA. Sixty-three of the isolates tested were race 1. The rest of the isolates were grouped as race 3. Thirty-one of the 42 isolates lacked the *ToxA* gene. Five of the tested wheat genotypes were susceptible to both race 3 and Ark Lon B2, while 4 were susceptible to Ark Lon B2 but not to race 3. Similarly, four wheat genotypes were susceptible to race 3 but not to the isolate Ark Lon B2. All 20 wheat genotypes were insensitive to Ptr Tox A and resistant to race 5.

Pathogenic and genotypic analysis among Iranian isolates of *Macrophomina phaseolina*

N. ALLAGHEBANDZADEH (2), S. Rezaee (2), B. Mahmoudi (1), H. Zamani Zadeh (2)
(1) Sugar Beet Seed Institute, Karaj, Tehran, Iran; (2) Science and Research Branch, Islamic Azad University, Tehran, Tehran, Iran
Phytopathology 98:S11

Macrophomina phaseolina is one of the soil borne plant pathogens in the tropics and subtropics with a wide host range. The pathogen causes root rot of sugar beet in different regions of Iran. In order to study of pathogenic variability and genetic diversity of the pathogen, 16 isolates of *M. phaseolina* from sugar beet, soybean and sesame fields were collected from different regions of Iran. Pathogenic variability was evaluated through *in vitro* and green house conditions. The results revealed considerable variation in aggressiveness of the isolates. Cluster analysis of the isolates classified them into three categories: highly, moderate and weakly virulent groups. Eleven random primers were used to amplify the total DNA of these isolates. The results of RAPD assay distinguished the isolates into five groups on the basis of pathogenicity, host and origin.

Characterization of Abutilon yellow mosaic virus, a tobamovirus occurring in flowering maple (*Abutilon* × *hybrida*)
C. V. ALMEYDA-BECERRA (1), B. E. Lockhart (1)
(1) University of Minnesota
Phytopathology 98:S12

A mosaic disease of flowering maple *Abutilon* × *hybrida* observed in Minnesota in 2007 was determined to be caused by a tobamovirus. This is the first report of a tobamovirus infecting *Abutilon*. It was named Abutilon yellow mosaic virus (AbYMV) and closely resembled *Hibiscus latent Fort Pierce virus* (HLFPV). These two tobamoviruses are serologically indistinguishable and have a high degree of sequence identity in the coat protein (99%), movement protein (97%) and in the 5093 bp sequence (99%) representing approximately 80% of the complete viral genome. However, they differ significantly in host range. The only significant sequence difference between AbYMV and HLFPV occurs close to the 3′ end of ORF1 where the amino acid sequence is EGFFSD in AbYMV and the sequence of the corresponding region in HLFVP is KGV•AE. It is possible, but remains to be shown, that the significant difference in host range between the two viruses may be linked to this minor change in sequence.

Characterization of Tomato yellow blotch virus, a new tymovirus occurring in greenhouse-grown tomatoes in Minnesota
C. V. ALMEYDA-BECERRA (1), B. E. Lockhart (1)
(1) University of Minnesota
Phytopathology 98:S12

A tymovirus occurring in greenhouse-grown tomatoes in Minnesota was characterized in 2007 and was named provisionally Tomato yellow blotch virus (ToYBV) after its characteristic foliar symptoms. ToYBV is most closely related to *Physalis mottle virus* (PhMV) in coat protein (85%) and total genome (80%) nucleotide sequence. Based on these genome sequence differences and consistent differences in host range and symptomatology, ToYBV is proposed as a new member of the genus *Tymovirus* distinct from PhMV. A related virus named Physalis mosaic virus was reported from Illinois, but there is insufficient information to determine its possible relationship to ToYBV and PhMV.

veA* and *laeA* interactions in *Aspergillus flavus
S. AMAIKE (1), J. Bok (1), N. P. Keller (1)
(1) University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 98:S12

Aspergillus flavus, a mycotoxigenic filamentous fungus, occurs as a saprophyte in soils worldwide and also colonizes several important agricultural crops, such as corn and peanuts. The pathogen can produce the carcinogen, aflatoxin, in these crops leading to economic and health concerns. Two genes, *veA* and *laeA*, have recently been found to regulate developmental processes in *A. flavus* including sclerotial, conidial and aflatoxin production. Deletion of both genes in *A. nidulans* yield similar phenotypes, possibly suggesting a connection between the two proteins. To learn more about *veA* and *laeA* functions in *A. flavus*, we created several A. flavus isogenic mutants differing only in copy number of *veA* and *laeA* genes. Those strains include wildtype, delta*veA*, delta*laeA*, overexpression *laeA* (*OE::laeA*), overexpression *veA* (*OE::veA*) and the double OE mutant, *OE::veA*; *OE::laeA*. Each strain will be tested in sclerotial, conidial and aflatoxin production and a summary of their phenotypes and pathogenicity attributes will be presented.

Monitoring resistance in *Monilinia fructicola* populations in the southeastern United States for enhanced brown rot control in peach
A. AMIRI (1), P. Brannen (2), G. Schnabel (1)
(1) Clemson University, Clemson, SC, USA; (2) University of Georgia, Athens, GA
Phytopathology 98:S12

A bio-assay consisting of a lipbalm tube filled with potato dextrose agar (PDA) was developed for on-farm assessment of resistance in *M. fructicola* to sterol demethylation inhibitor (DMI), benzimidazole (BZI) and quinone outside inhibitor (QoI) fungicides. A field survey conducted to compare the lipbalm tube to the traditional Petri dish revealed a strong correlation (R = 0.96) between the two methods in assessing resistance in *M. fructicola*. A total of 200 *M. fructicola* isolates were collected in 2007 from seven peach orchards in South Carolina and three orchards in Georgia. Among 130 isolates collected in South Carolina, 22.7, 21.2, and 36.4% grew on PDA amended with discriminatory doses of 0.3, 50.0, and 1.0 µg/ml of the DMI propiconazole, the BZI thiophanate methyl and the QoI azoxystrobin, respectively. Among 70 isolates collected in Georgia, 39.5, 5.7, and 60.0% grew at the above respective doses. The majority of 25 isolates growing at 1 µg/ml azoxystrobin exhibited more than 20% relative growth at 5 µg/ml azoxystrobin. These isolates were not controlled by the label rate of formulated azoxystrobin (Abound) in detached fruit assays. Our results show the potential application of the lipbalm tube method for local on-farm resistance monitoring. This survey also shows persistence of BZI resistance, emerging DMI resistance in this part of the country and suggests development of reduced sensitivity to QoIs in *M. fructicola* populations.

Development of a selective medium for recovery of *Monilinia fructicola* from peach fruit
A. AMIRI (1), P. M. Brannen (2), G. Schnabel (1)
(1) Clemson University, Clemson, SC; (2) University of Georgia, Athens, GA
Phytopathology 98:S12

A selective medium was developed for the recovery of *M. fructicola* from peach fruit. The fungicides fosetyl-Al, dichloran, ammonium molybdate, and 2-deoxy-D-glucose were tested in potato dextrose agar (PDA) for their activity against *M. fructicola* and seven common fungal contaminants of peach, including *Alternaria alternata*, *Aspergillus niger*, *Colletotrichum acutatum*, *Gilbertella persicaria*, *Penicillium expansum*, *Phomopsis amygdali*, and *Rhizopus stolonifer*. Dichloran, ammonium molybdate, and 2-deoxy-D-glucose inhibited mycelial growth and spore germination of all test fungi including *M. fructicola* at comparable levels. Fosetyl-Al added to PDA at 500 or 1000 µg/ml (PDA-F) did not inhibit germination of any of the fungi but had a strong effect on mycelial growth of six out of eight test fungi at 1000 µg/ml exceptions being *R. stolonifer* and *M. fructicola*. Fosetyl-Al added to acidified PDA at 500 µg/ml (APDA-F500) inhibited germination of *A. alternata* and *P. amygdali* and impaired germ tube growth of four other fungi including *R. stolinifer*, but not *M. fructicola*. Percent recovery of *M. fructicola* from peach fruit was higher on APDA-F500 (17 and 69% in July and August, respectively) compared to PDA-F500 medium (3.5 and 50% for the same periods, respectively). Our results indicate that APDA-F500 is a suitable medium for *M. fructicola* isolations.

Basipetal translocation of propiconazole following trunk infusion of peach trees
A. AMIRI (1), K. E. Bussey (2), M. B. Riley (1), G. Schnabel (1)
(1) Clemson University, Clemson, SC; (2) North Carolina State University, Raleigh, NC
Phytopathology 98:S12

In the Southeastern United States, Armillaria root rot (ARR) is caused mainly by *Armillaria tabescens*, and constitutes one of the greatest threats to peach production in the region. Propiconazole, a sterol demethylation inhibitor fungicide, was found highly effective in inhibiting mycelial growth of two *A. tabescens* isolates *in vitro* (average EC_{50} value of 0.6 µg/ml) compared to fungicides from five other chemical classes. Formulated propiconazole was infused into the trunk of peach trees in the spring, summer, and fall of 2005 and 2006 using a non-pressurized infusion system. Propiconazole concentrations were determined in trunk sections above and below the infusion site and in primary roots using gas chromatography-mass spectrometry. The fungicide accumulated consistently in primary peach roots following spring and fall infusions. The highest levels were detected after fall infusions (9.2 µg/g in 2005 and 6.7 µg/g in 2006), while spring infusions resulted in lower propiconazole concentrations (1.7 µg/g in 2005 and 5.6 µg/g in 2006). Propiconazole was also detected in trunk sections both from above and below the infusion site (between 1.7 and 170 µg/g). Our findings support previously published evidence suggesting that trunk infusion of propiconazole may be useful for targeted ARR management.

Comparative analysis of genes involved in resting structure development in *Verticillium dahliae* and *V. albo-atrum*
S. G. AMYOTTE (2), K. F. Dobinson (1)
(1) Agriculture & Agri-Food Canada, London, Ontario, Canada; (2) University of Western Ontario, London, Ontario, Canada
Phytopathology 98:S12

The soilborne filamentous fungi *Verticillium dahliae* (Kleb.) and *V. albo-atrum* (Reinke et Berth.) are the causal agents of an economically significant vascular wilt disease which affects a wide range of crops and woody plants in temperate climates. Verticillium wilt is difficult to control largely because of the production by these fungi of environmentally resistant resting structures. *V. dahliae* produces structures called microsclerotia (MCS), while the closely related *V. albo-atrum* produces dark resting mycelia (DRM). A *V. dahliae* MAPK gene (*VMK1*) and a hydrophobin gene (*VDH1*) were previously shown to be important for MCS production. In *V. albo-atrum*, we have identified an orthologue of *VMK1* (*VaVMK1*) and *VDH1* (*VaVDH1*), and have initiated a comparative study between the *V. dahliae* and *V. albo-atrum* orthologues. *V. albo-atrum* disruption mutants of *VaVMK1* and *VaVDH1* were produced. In the *VaVMK1* mutants, DRM development was severely limited, suggesting a conserved role for the MAPK in resting structure development. However, disruption of *VaVDH1* had no effect on DRM production. Northern hybridization analysis revealed that *VaVDH1* in *V. albo-atrum* has a different temporal expression pattern than *VDH1* has in *V. dahliae*. The sequences upstream of *VDH1* and *VaVDH1*, which contain the promoter elements of

these two genes, are only 86% identical. Therefore, to determine if differences in the promoter sequences contribute to the different temporal expression patterns, in V. *dahliae* we are replacing the *VDH1* gene and 5′ flanking sequences with those of *VaVDH1*. Results are expected to contribute to a better understanding of the role these MAPK and hydrophobin genes have in resting structure development in *V. dahliae* and *V. albo-atrum*.

The nation's first agricultural experiment station: Discoveries that shaped plant pathology

S. L. Anagnostakis (1), S. DOUGLAS (1)
(1) The Connecticut Agricultural Experiment Station, New Haven, CT, USA
Phytopathology 98:S13

The Connecticut Agricultural Experiment Station was established in 1875 "for the purpose of promoting agriculture by scientific investigation and experiment." With new funds made available by the Hatch Act in 1887, the Station hired Dr. Roland Thaxter, a student of Farlow at Harvard, and established the Mycology Department in a new building, which Thaxter called his mycotheca, or "fungus box." Among Thaxter's accomplishments while at the Station were proving the etiology of potato scab and devising a knapsack sprayer for fungicide trials, ostensibly from his wife's wash boiler. Research on plant pathology has continued at the Station, as the name of the department changed from Botany, to Plant Pathology and Botany, and to its current name, Plant Pathology and Ecology. Over the years, the Station contributed three presidents to the American Phytopathological Society and made many contributions that have shaped the science of Plant Pathology. This poster highlights some of these contributions from past to present.

Whole genome response of Agrobacterium to Acetosyringone: A phenolic inducer

A. ANAND (1), Z. Vaghchhipawala (1), Y. Tang (1), K. Mysore (1)
(1) Samuel Roberts Noble Foundation
Phytopathology 98:S13

Agrobacterium tumefaciens is a phytopathogen that causes crown gall disease on a wide variety of plants. One key process in *Agrobacterium*-plant interaction involves the induction of virulence (vir) genes, which are not expressed in saprophytic bacteria. It is thought that vir genes are induced by plant-released signals, which include specific phenolic compounds, monosaccharides, acidic pH and temperatures below 30°C. Two phenolic compounds, acetosyringone (AS) and alpha-hydroxyacetosyringone, were the first two compounds identified as specific vir gene inducers. AS, is also commonly used in culture media to induce *Agrobacterium*. Presently, there is no study that has completely characterized the direct effects of phenolic compounds on the whole bacterial genome. Using *Agrobacterium* microarrays, we recently reported that phytohormone salicylic acid attenuates *Agrobacterium* virulence by interfering with the induction of vir genes. The transcriptome profiling of *Agrobacterium* in response to AS, and functional analyses of selected genes will be presented.

c-diGMP regulation of *Xylella fastidiosa* Temecula gene expression and biofilm formation

V. ANCONA (2), S. Wei (2), D. Appel (2), Y. Hayakawa (1), P. J. DeFigueiredo (2)
(1) Nagoya University; (2) Texas A&M University, College Station, TX, USA
Phytopathology 98:S13

Xylella fastidiosa (*Xf*) is a Gram-negative non-flagellated bacterium that causes a number of economically important plant diseases, including Pierce's disease of grapevine, which has placed the wine industries of California, Texas and other states at risk. To date the molecular virulence mechanisms of *Xf* remain obscure. Our project focuses on characterization of the role that the cyclic diguanylate (d-diGMP) signaling system plays in mediating biofilm formation and virulence of *Xf* to enable a strategy for Pierce's disease control. c-diGMP is a bacterial second messenger that regulates cell-cell signaling, biofilm formation, motility, and virulence in human and plant pathogenic bacteria. This molecule is produced from two molecules of GTP by diguanylate cyclase enzymes (DGCs). DGC activity resides in the GGDEF (Gly-Gly-Asp-Glu-Phe) domain of these proteins. c-di–GMP is degraded to GMP, via the linear pGpG by phosphodiesterases (PDEs). PDE activity has been show to reside proteins containing EAL (Glu-Ala-Leu) or HD-GYP (His-Asp, Gly-Tyr-Pro) domains. The interplay of DGC and PDE activities controls intracellular c-di-GMP concentration and hence c-di-GMP signaling. In addition, a PilZ domain, which is thought to possess c-di-GMP binding activity, has recently been described and believed to regulate downstream events including biofilm formation, motility and virulence. BLAST analysis revealed 6 proteins containing GGDEF domains, EAL domains and/or HD-GYP domains and 2 proteins with PilZ domains. We will present results of gene expression changes in response to c-di-GMP treatment of these genes and from additional genes involved in bacterial motility, attachment, and signaling using real-time PCR methods. We will also present results from the effect of c-di-GMP on biofilm formation of *Xf* with mutations on genes directly involved in the c-diGMP pathway.

Towards positional cloning of an avirulence gene from *Cronartium quercuum* f. sp. *fusiforme*

C. ANDERSON (1), J. A. Smith (1), J. M. Davis (1), T. L. Kubisiak (2), C. Nelson (2)
(1) School of Forest Resources and Conservation, University of Florida, Gainesville, FL 32611, USA; (2) Southern Institute of Forest Genetics, U.S. Forest Service, Saucier, MS 39574, USA
Phytopathology 98:S13

Cronartium quercuum f. sp. *fusiforme* (*Cqf*) is the causal agent of fusiform rust on oaks and pines and is the most devastating fungal pathogen on pine trees in southeastern USA, with losses estimated to exceed $135 million annually. Genetic resistance is the only economically feasible control method for this pathogen and several resistance genes have been identified and utilized in breeding programs. However, as pine resistance genes function by recognition of specifically corresponding avirulence genes in the pathogen, successful deployment of resistant varieties will only occur if the corresponding avirulence gene is present at high frequency in the local pathogen population. The *Cqf* avirulence gene, *AvrFr1*, specifically interacts with the *Fr1* resistance gene in loblolly pine and a project has been initiated to isolate *AvrFr1* by positional cloning. Four Randomly Amplified Polymorphic DNA (RAPD) markers have been identified that are linked to the avirulence locus and these define the location of *AvrFr1* within a 10.4 cM interval. Fluorescent Amplified Fragment Length Polymorphism (AFLP) technology is now being used to fine map the location of *AvrFr1* within this interval, to facilitate our positional cloning efforts. It is hoped that the isolation of *AvrFr1* and other *Cqf* avirulence genes will ultimately aid in deployment of resistant pine genotypes by allowing the frequencies of avirulence alleles to be determined in natural *Cqf* populations.

Historical look at aspen management and genetic improvement for Hypoxylon canker resistance

N. A. ANDERSON (2), M. E. Ostry (1)
(1) USDA Forest Service, St. Paul, MN, USA; (2) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S13

Hypoxylon canker caused by *Entoleuca mammata* is the most damaging disease of aspen (*Populus tremuloides*) in the Lake States during the first 20–30 years of stand establishment. Most studies on host resistance involved post-infection factors. We have found that pre-infection factors such as early response to wounds and stand structure are equally important. The biochemical parameters for infection were known prior to the discovery of the importance of insect oviposition wounds as entry courts. The biochemistry and infection biology have contributed to the disease complexity and difficulty in finding resistance. Spatial resistance, a function of high stem stocking in young stands, promotes self-pruning and discourages oviposition by several insect species requiring high levels of sunlight. These findings support the recommendation that managers maintain young aspen stands at maximum density. In our long-term genetic studies most progeny of randomly selected disease-free parents were just as susceptible as progeny from diseased parents when planted at a wide spacing. However, a few disease-free progeny were able to wall-off insect oviposition wounds and have superior growth and form. These individuals, only identified after 40 years, would have value as parents in a breeding program. Resistance to infection of improved aspen may be durable because a somatic incompatibility system in the pathogen does not allow pathogenic races to develop. The first 15–20 year rotation of plantations of widely-spaced aspen would benefit if they were established with resistant progeny from long-term tests of clones. After coppice reproduction spatial resistance will also reduce disease incidence.

Isolation and functional characterisation of a cluster of TIR-NBS-LRR genes linked to powdery mildew resistance in grapevine

C. Anderson (1), A. Feechan (1), A. M. Jermakow (1), A. Bouquet (3), A. Adam-Blondon (2), M. R. Thomas (1), I. B. DRY (1)
(1) CSIRO Plant Industry, PO Box 350, Glen Osmond SA 5064, Australia; (2) INRA-URGV 2, rue Gaston Cremieux CP 5708 F-91057 Evry, France; (3) UMR BEPC, Campus Agro-M/INRA, 2 place Viala, 34060, Montpellier, France
Phytopathology 98:S13

Grapevine powdery mildew, caused by *Erysiphe necator* (syn. *Uncinula necator*), is an economically important fungal disease of grapevines worldwide causing reduced yield and loss of quality. The North American grapevine *Muscadinia rotundifolia* is completely resistant to powdery mildew due to the action of a single dominant locus designated *Run*1. Phenotypically, *Run*1 mediated resistance resembles that conferred by the barley powdery mildew resistance gene, *Mla*12, inducing a hypersensitive response, within infected epidermal cells, following fungal haustorium formation. The *Run*1 locus was introgressed into *V. vinifera* by successive back-crosses generating

segregating populations that were used in genetic and physical mapping studies. *Run*1 resistance has been localised to a region containing a cluster of eleven closely related resistance gene analogs (RGAs) encoding both full-length and truncated TIR-NBS-LRR proteins. Real-time expression analysis indicates differential expression of these RGA candidates in response to powdery mildew infection. Full-length (11–15 kb) genomic cassettes containing RGA candidates with native promoter and terminator sequences were agroinfiltrated into tobacco and confirmed to be transcriptionally active. Interestingly, one RGA candidate was found to be auto-active in tobacco causing necrotic lesions to form after 3–4 days. Analysis of the translated protein product of this RGA gene indicates it to have a deletion within the C-terminal domain compared to the other RGA genes suggesting that this region may be important for repression of wild-type RGA activity.

Biological effects of tombusvirus P19 and P22 proteins agroinfiltrated in *Nicotiana* species

C. A. ANGEL (2), Y. Hsieh (1), J. E. Schoelz (2)
(1) Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA; (2) Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA
Phytopathology 98:S14

The tomato bushy stunt virus (TBSV) P22 and P19 proteins play an important role in elicitation of symptoms and in triggering plant defenses in *Nicotiana* species. The TBSV P22 protein induces a hypersensitive response (HR) in *Nicotiana edwardsonii* and *N. glutinosa*, whereas the TBSV P19 protein activates an HR in *N. tabacum*. In addition, P19 is a silencing suppressor. In the present study, we have utilized an agroinfiltration assay to evaluate the capacity of the P19 and P22 proteins of cucumber necrosis virus (CNV) and cymbidium ringspot virus (CymRSV) to trigger necrosis in *Nicotiana* species. In contrast to TBSV P19, the P19 proteins of CNV and CymRSV did not elicit necrosis in *N. tabacum*, although they did function as silencing suppressors in transient co-agroinfiltration assays with green fluorescent protein expressing constructs. Results obtained with the CNV P22 protein were similar to those for TBSV P22, as the CNV P22 protein elicited necrosis in *N. edwardsonii* at the same time as TBSV P22 when these proteins were agroinfiltrated into *N. edwardsonii*. However, in these same tests the symptom induction by the CymRSV P22 protein was slower and more variable. We conclude that *Nicotiana* species are able to perceive subtle differences between the P22 and P19 proteins of TBSV, CNV, and CymRSV, which affects the plant's response to these proteins.

Big vein disease of lettuce: Relationship between symptom intensity and viral RNA accumulation

C. Araya (1), L. Roman (1), E. Peña (1), R. Mora (1), I. ROSALES (1)
(1) Instituto de Investigaciones Agropecuarias (Inia), Plant Breeding and Biotechnology Department, Santiago, Rm, Chile
Phytopathology 98:S14

Big vein disease (BVD) was first described in Chile in 2003, and it is now widely distributed in the country, with an overall incidence of over 50%. Typical symptoms associated to BVD are chlorotic clearing around the leaf veins, leaf distortion and reduction of the head size. A viral etiology has been described for this disease involving two different viruses: Mirafiori lettuce big-vein virus (MLBVV) and Lettuce big-vein associated virus (LBVaV). In this work the relationship between symptom intensity and viral RNA accumulation was studied. For this study, lettuces from 14 different varieties belonging to two different types (cos and iceberg) were collected in an experimental field located in Santiago, RM. The plants were classified according to its symptomatology as mild, moderate or severe. The viral coat protein (CP) concentration of the two viruses was evaluated by DAS-ELISA and the accumulation of the viral RNAs that encode for these proteins by semiquantitative reverse-transcription (sqRT-PCR). The sqRT-PCR revealed that the MLBVV-RNA-3 accumulation was two times higher in the iceberg than the cos varieties ($P < 0.05$). However, for LBVaV, RNA-2 accumulation was higher in the varieties belonging to the cos type ($P < 0.05$). The results also indicate that there are no statistically significant differences ($P > 0.05$) in the viral RNA accumulation from plants that were classified in the different symptom intensity groups mentioned above. These results suggested that does not exist a direct relationship between symptom intensity and accumulation of the viral RNAs encoding for the CP of MLBVV or LBVaV in this plant-virus interaction. This research was supported by projects: FIA-PI-C-2005-1-A-051 and FONDECYT INICIACIÓN Nº11060173.

Reaction of wild watermelon germplasm to southern root-knot nematode in South Carolina

J. J. ARISS (2), J. A. Thies (2), C. S. Kousik (2), R. L. Hassell (1)
(1) Coastal Research and Education Center, Clemson University, Charleston, SC; (2) U.S. Vegetable Laboratory, USDA-ARS, Charleston, SC
Phytopathology 98:S14

Southern root-knot nematode (*Meloidogyne incognita*, RKN) can be a major limiting factor in watermelon (*Citrullus lanatus* var. *lanatus*) production in southern regions of the U.S. Wild watermelon (*Citrullus lanatus* var. *citroides*) populations have been identified as having higher resistance to RKN than commercial watermelon in greenhouse studies. Selected wild watermelon populations showing varying levels of RKN-resistance were further evaluated in an artificially infested field site in 2006 and 2007. In 2006, 19 experimental germplasm lines derived from USDA-ARS Germplasm Resources Information Network plant introduction accessions were compared with susceptible check populations. Two wild watermelon populations exhibited significantly less root galling than susceptible check populations and three additional populations were identified as possessing other favorable resistance traits. These five best performing wild watermelon experimental lines were evaluated at the same field site with and without pre-plant methyl bromide fumigation in 2007. In general, the experimental lines performed similarly with and without methyl bromide fumigation in regard to RKN resistance characteristics. Overall, the five *Citrullus lanatus* var. *citroides* populations exhibited less root galling, reduced RKN reproduction, and increased root vigor compared to susceptible checks. Results from these two studies suggest wild watermelon populations may prove a useful source of resistance to RKN, either as breeding material or as rootstock for commercial watermelon cultivars.

Effect of fungus gnat *Bradysia impatiens* (Diptera: Sciaridae) feeding on subsequent *Pythium aphanidermatum* infection of geranium seedlings (*Pelargonium* × *hortorum*)

S. E. ARNOLD (1), E. B. Nelson (2), J. P. Sanderson (1), M. L. Daughtrey (2), S. P. Wraight (3)
(1) Cornell University, Department of Entomology, Ithaca, NY, USA; (2) Cornell University, Department of Plant Pathology, Ithaca, NY, USA; (3) USDA-ARS, Robert W. Holley Center for Agriculture & Health, Ithaca, NY, USA
Phytopathology 98:S14

Dark-winged fungus gnats in the genus *Bradysia* (Diptera: Sciaridae) and root rot pathogens in the genus *Pythium* (Oomycetes) are important pests of greenhouse floriculture. Observations have pointed to a possible correlation between Pythium root rot disease and fungus gnat infestations; however, interactions among fungus gnats, *Pythium* spp. and floral crops have not been thoroughly investigated. The goal of this study was to determine the effects of plant feeding by fungus gnat larvae on subsequent *Pythium aphanidermatum* (*Pa*) infection of geranium seedlings (*Pelargonium* × *hortorum*). A 2 × 2 factorial experiment with fungus gnat feeding damage and *Pa* inoculation as treatments was conducted using a laboratory bioassay. Geranium seeds were germinated on water-saturated filter paper in small Petri dishes (1 seed/dish). When the seedlings were 7 days old, 4th-instar fungus gnat larvae were placed in the dishes (3/dish) and allowed to feed on the seedlings. The larvae were removed after 24 h, and the dishes were inoculated by pipetting 0.4 ml of *Pa* zoospore suspension onto the filter paper. A total of 13 assays were conducted, using 80–100 seedlings/assay and inocula with ca. 25–500 zoospores/ml. Mortality of seedlings recorded 7 days post-inoculation increased significantly in the presence of *Pa* and with increasing zoospore concentration. In the fungus gnat + *Pa* treatments, there were highly significant reductions (up to 37.5 percentage points) in geranium seedling mortality compared to *Pa* inoculation alone. Further experiments are warranted to elucidate the mechanism of this interaction. These findings enhance our understanding of the association between fungus gnats and *Pythium* spp. in greenhouse floriculture.

Population biology of *Verticillium dahliae* isolates from lettuce in the Salinas Valley of California

Z. K. ATALLAH (2), K. Maruthachalam (2), R. J. Hayes (1), S. J. Klosterman (1), K. V. Subbarao (2)
(1) USDA-ARS, Salinas, CA; (2) University of California-Davis, Salinas, CA
Phytopathology 98:S14

Verticillium dahliae is a soilborne fungus and the primary causal agent of Verticillium wilt, which affects numerous crops worldwide. Many crops grown in the Salinas Valley (SV) of California, including lettuce (*Lactuca sativa*), are susceptible to *V. dahliae* and severe outbreaks are common in certain fields. Since 1995, *V. dahliae* has emerged as a serious threat to lettuce production. We documented two races in *V. dahliae* on lettuce in SV. Currently, there are no sources of race 2-resistant lettuce and costly soil fumigations with methyl-bromide are necessary for a successful production. Besides the presence of two races of *V. dahliae*, little is known about the population diversity of the pathogen in SV. We have sequenced the intergenic spacer region (IGS) of over 250 isolates collected from a variety of crops and weeds grown in SV. Clustering using IGS sequences parallels virulence on differential hosts. Additionally, we identified 100 simple sequence repeat (SSR, microsatellite) motifs in the genome of lettuce isolate VdLs.17. These SSRs include the range of motifs from di to deca-nucleotides. Primers were

generated to amplify the targeted SSR motifs, and SSR haplotypes from the isolate collection are currently being generated. Comparisons will be made with groupings generated by IGS sequences, AFLP genotyping and virulence analyses. Spatial population genetics studies of *V. dahliae* in lettuce fields will ensue to elucidate the population structure in SV.

Avirulence genes from *Xanthomonas axonopodis* pv. *glycines* causes specific genotype in soybean

D. ATHINUWAT (2), S. Prathuangwong (2), T. J. Burr (1)
(1) Department of Plant Pathology, College of Agriculture and Life Sciences, NYSAES, Cornell University, Geneva, NY 14456, USA; (2) Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bankok 10900, Thailand
Phytopathology 98:S15

The 34 strains of *Xanthomonas axonopodis* pv. *glycines* (*Xag*), isolated from different soybean cultivations, were determined for race-cultivar specific incompatibility on 3-recommended soybean cultivars including Williams82 (resistance), Spencer (susceptible), and P1 520733 (susceptible) by handle spray inoculation and infiltration assay at 1 × 108 cfu/ml population density. We identified three races of *Xag* strains based on their interactions on these soybean cultivars. Race1 (23 strains) induced hypersensitive response (HR) on Williams82 within 48 h and Race2 (7 strains) induced disease on three cultivars tested. Race3 (4 strains) elicited HR on resistant cultivar (Williams82) and disease induction on the two susceptible cultivars tested. *Xag* strain KU-P-SW005 from Race 3 had been made mutagenesis with the EZ::TNTM<KAN-2>Tnp TransposomeTM system (Epicenter, WI, USA). This transposome was introduced into *Xag* cells by electroporation. The KU-P-D005 mutant out of total 3,336 strains obtained from KU-P-SW005 mutagenesis showed the disease on three soybean cultivars. This mutant increased cellulase production and 20% bacterial pustule severity when compared with the wildtype. KU-P-D005 induced different disease symptoms and also HR on nonhosts (tomato and tobacco) that was different from wildtype. The KU-P-D005 sequence showed high identity (100% at nucleotide level) with avrb6 gene (PSTRU-3, accession number L06634) of *X. axonopodis* pv. *malvacearum*. This suggests ORF region of avr genes wildtype has been inserted with EZ::TN transposome. The characterization and cloning of *Xag* avr genes provided genetic evidence of gene-for-gene interaction in the bacterial pustule disease of soybean. Further characterization of the avr genes and subsequent R genes in soybean will be useful for developing new method to control pustule disease.

Effect of regulatory genes on the production of volatiles by Pseudomonas chlororaphis PA23 and identification of antifungal antibiotics of Bacillus species using polymerase chain reaction and MALDI-TOF mass spectrometry

S. N. Athukorala (3), D. FERNANDO (3), K. Rashid (1), T. de Kievit (2)
(1) Agriculture and Agri-Food Canada, 100 Morden, Manitoba, Canada; (2) Dept. of Microbiology, University of Manitoba, Winnipeg, MB R3T 2N2, Canada; (3) Dept. of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
Phytopathology 98:S15

Effect of regulatory genes on the production of three antimicrobial volatiles (nonanal, benzothiazole, 2-ethyl-1-hexanol) produced by Pseudomonas chlororaphis strain PA23 inhibitory to Sclerotinia sclerotiorum and the presence of antibiotic biosynthetic genes (bacillomycin D, iturin A, surfactin, mycosubtilin, fengycin and zwittermicin A) in 21 Bacillus sp. showing mycelial inhibition of S. sclerotiorum were investigated. Tn mutants (PA23-314, PA23-314 pUCP23, PA23-314 gacS, PA23-63, PA23-754, PA23-443 and PA23-443 PtrA) of wild-type strain PA23 was tested for their ability to inhibit the mycelial growth, sclerotial germination and ascospore germination of S. sclerotiorum and the production of volatile compounds. PA23, PA23-314, PA23-314 gacS, PA23-63, PA23-754 and PA23-443 ptrA showed a significant reduction of mycelial growth and sclerotial germination. Except for PA23-314 and PA23-314 pUCP23, all other strains inhibited ascospore germination completely after 48 h. GC/MS showed all strains produced nonanal, benzothiazole, 2-ethyl-1-hexanol. PCR amplification of biosynthetic genes of Bacillus sp. showed the presence biosynthetic genes for surfactin (21/21) and iturin A (20/21). B. subtilis 3057, B. amyloliquefaciens BS6, B. mycoides 4079 were positive for bacillomycin D while B. subtilis H-08-02, B. subtilis 3057, B. amyloliquefaciens BS6, B. mycoides 4079 showed presence of fengycin biosynthetic gene. The zwittermicin A gene was detected in B. mycoides S, B. thuringiensis BS8 and B. amyloliquefaciens BS6. Production of particular antibiotics in strains BS6, H-08-02, 3057 and 4079 was confirmed through MALDI-TOF.

A real-time PCR assay for the detection of *Pasteuria nishizawae* in soil

N. ATIBALENTJA (2), M. Babadoost (2), G. R. Noel (1)
(1) USDA-ARS, Urbana, IL 61801; (2) University of Illinois, Urbana, IL 61801
Phytopathology 98:S15

Pasteuria nishizawae is a mycelial, endospore-forming, and obligately parasitic bacterium that has shown a great potential for the biological control of the soybean cyst nematode, *Heterodera glycines*, in both microplot and field studies. Other than China, Japan, and South Korea, *P. nishizawae* has been found naturally infecting *H. glycines* only in the United States (U.S.) at Urbana, Illinois. It is not known whether the bacterium occurs in other soybean fields in Illinois or elsewhere in the U.S., especially in fields where soil suppressiveness has been observed. Addressing this question requires development of a reliable assay to determine the presence, population density, and distribution of *P. nishizawae* in soybean fields. The current method that consists in counting the number of endospores attached to second-stage juveniles (J2) of *H. glycines* is not appropriate for that goal. We have developed a real-time quantitative PCR assay that is based on the 16S rRNA gene sequence of *P. nishizawae*. The assay has been successfully used to detect minute amounts (less than 1,000 copies) of the target from a crude DNA extract. This assay, when improved, will be very useful for conducting surveys to determine whether *P. nishizawae* occurs in soybean fields in the U.S.

Taxonomic complexity of powdery mildew pathogens found on lentil and pea in the U.S. Pacific Northwest

R. P. ATTANAYAKE (3), D. Glawe (4), K. McPhee (1), F. Dugan (1), W. Chen (2)
(1) USDA ARS, Pullman, WA, USA; (2) USDA ARS, Washington State University; (3) Washington State University, Pullman, WA, USA; (4) Washington State University, and University of Washington, Seattle, WA, USA
Phytopathology 98:S15

Classification of powdery mildews found on lentil and pea in greenhouse and field production conditions in the U.S. Pacific Northwest was investigated using morphological and molecular characters. Isolates collected from lentil plants grown in the greenhouse or field displayed morphologies in substantial agreement with descriptions of *Erysiphe trifolii*, but sometimes with more extensively branched chasmothecial appendages resembling those of *E. diffusa*. ITS sequences of the lentil fungi were identical to each other, and more similar to GenBank accessions of *E. trifolii* (99.5%) than of E. diffusa (97%). The data suggest there may be more than one Erysiphe species causing lentil powdery mildew. The fungus on field-grown pea plants was determined to be E. pisi. However, powdery mildew samples obtained from greenhouse pea plants were either *E. pisi* or *E. trofolii* depending on the time of sampling and greenhouse location. Therefore, the powdery mildews infecting lentil and pea are more diverse than previously assumed. Powdery mildews from black medic (*Medicago* sp.) and sweet clover (*Melilotus* sp.) found near the greenhouses exhibited ITS sequences with 99.9 to 100% similarity to isolates from lentil and pea in the greenhouses, and to isolates from lentil from the field. These weedy legumes could be inoculum sources for powdery mildew of lentil and pea in the greenhouses, and serve as alternative hosts for cultivated legumes.

Impact of sunlight and its components on severity of grapevine powdery mildew

C. N. AUSTIN (1), A. N. Lakso (2), R. C. Seem (1), D. G. Reigel (1), D. M. Gadoury (1), W. F. Wilcox (1)
(1) Cornell University, Department of Plant Pathology and Plant-Microbe Biology NYSAES, Geneva, NY, USA; (2) Horticultural Science NYSAES, Geneva, NY, USA
Phytopathology 98:S15

Grapevine powdery mildew (Uncinula [Erysiphe] necator) is often more severe in shaded portions of a vineyard compared to those in full sunlight. We have shown that this is due to increased UV radiation, and, at higher air temperatures, the surface of sun-exposed tissues can be substantially elevated into a deleterious or lethal range for the pathogen. UV levels in shade ranged from 3–20% of those in direct sun, and mid-day temperatures of sun-exposed leaves were 5–15°C higher than shaded leaves. By modifying the vineyard canopy to promote light penetration, we have decreased severity of powdery mildew on fruit by up to 50%, and on leaves by up to 87%. Controlled environment experiments indicated that UV exposure combined with the high temperatures dramatically reduced germ tube and appressorium formation of conidia. Controlled environment experiments indicated that UV exposure combined with the high temperatures dramatically reduced germ tube and appressorium formation of conidia. However, some conidia still reacted positively to the vital stain fluorescein diacetate after UV exposure, and produced colonies when transferred to healthy leaves. Thus, at some level the UV treatments were fungistatic as well as fungicidal.

The importance of *Fusarium* and *Pythium* species in seed decay and root rot on soybean

M. V. AVANZATO (1), J. C. Rupe (1), C. S. Rothrock (1)
(1) University of Arkansas
Phytopathology 98:S16

The importance of *Pythium* spp. in seed and root rot symptoms on soybean seedlings has been well documented, but information about individual species of *Fusarium* on seeds and roots is limited. The objective of this research was to evaluate the role of *P. irregulare*, *P. mamillatum* and the *Fusarium* spp.: *F. tricinctum*, *F. oxysporum* and *F. equiseti* on seedling establishment. All the isolates evaluated were recovered from soybean roots or seeds grown in a naturally infested field soil from Hope, Arkansas, under controlled environmental conditions. The pathogenicity of individual isolates of *Fusarium* and *Pythium* was determined in controlled environmental conditions using artificially infected soilless potting media at 23°C. A seed germination assay also was conducted at room temperature with *Fusarium* spp. Results showed a wide range in plant stands but little root discoloration, among *Fusarium* isolates. *Pythium* isolates also showed differences in plant stands, but root discoloration was more severe than for *Fusarium* isolates. Screening for *Fusarium* pathogenicity by the seed germination assay was not comparable to the soil infestation technique. Interaction between isolates of *Fusarium* spp. and *Pythium* spp. on soybean is being investigated using a soil infestation technique.

Molecular characterization of *Alternaria alternata* field isolates highly resistant to the carboxamide fungicide boscalid

H. F. AVENOT (3), A. Sellam (2), D. Morgan (3), G. Karaoglanidis (1), T. J. Michailides (3)
(1) Aristotelian University of Thessaloniki, Thessaloniki, Greece; (2) Biotechnology Research Institute, Montreal, Canada; (3) University of California Davis, Kearney Agricultural Center, Parlier, CA, USA
Phytopathology 98:S16

Thirty-eight isolates of *Alternaria alternata* isolated from pistachio orchards with a history of Pristine® (pyraclostrobin + boscalid) applications displayed high levels of resistance to boscalid fungicide (mean EC_{50} values > 100 µg/ml) as determined with mycelial growth tests. A cross-resistance study revealed that the same isolates were also resistant to carboxin, a known inhibitor of succinate dehydrogenase (Sdh). To determine the genetic basis of boscalid resistance in *A. alternata*, the entire iron sulphur gene (*AaSdhB*) was isolated from a fungicide-sensitive isolate. The deduced amino-acid sequence showed high similarity with iron sulphur proteins (Ip) from other organisms. Comparison of full sequences of *AaSdhB* from sensitive and resistant isolates revealed that a highly conserved histidine residue (codon CAC in sensitive isolates) was converted to either tyrosine (codon TAC, in ten resistant isolates, type I mutants) or arginine (codon CGC, five resistant isolates type II mutants) at position 277. This finding is consistent with previously published reports, highlighting the crucial role of the conserved histidine residue. Twenty-three other resistant isolates (type III mutants) had no mutation in the histidine codon. The point mutation detected in type I mutants was used to design a pair of allele-specific PCR primers to facilitate rapid detection. A PCR-RFLP assay in which amplified gene fragments were digested with *Aci*I was successfully employed for the diagnosis of type II mutants. These molecular assays will greatly improve the detection and efficient monitoring of boscalid resistant *A. alternata* from pistachio.

A single amino-acid change in the cytochrome b560 subunit of succinate dehydrogenase complex (SdhC) correlates with boscalid resistance in *Alternaria alternata* isolates from California pistachio

H. F. AVENOT (2), A. Sellam (1), D. P. Morgan (2), T. J. Michailides (2)
(1) Biotechnology Research Institute, Montreal, Canada; (2) University of California Davis, Kearney Agricultural Center, Parlier, CA, USA
Phytopathology 98:S16

Alternaria late blight of pistachio caused by *Alternaria* spp. in the *alternata*, *tenuissima*, and *arborescens* species-groups is one of the most common fungal diseases of pistachio in California. Control of the disease requires multiple fungicide sprays. As a strategy to manage the widespread resistance to QoIs of *Alternaria* spp. in California pistachio orchards, the boscalid-containing fungicide Pristine® was registered for use against Alternaria late blight of pistachio, but reductions of its efficacy in the field have prompted concerns over development of resistance to boscalid. Studies carried out to test the control efficacy of boscalid revealed the existence of *A. alternata* isolates expressing high level of resistance to this fungicide (mean EC_{50} values > 100 µg/ml). The same isolates were also cross-resistant to carboxin, a known inhibitor of succinate dehydrogenase (Sdh). Studies with resistant mutants of other fungi to boscalid-analogs have shown that mutations in the B, C, and D subunits of the Sdh complex, the targets of boscalid, are involved in carboxamide resistance acquisition. To determine the genetic basis of boscalid resistance in *A. alternata*, the *A. alternata SdhC* gene (*AaSdhC*) was isolated from a fungicide-sensitive isolate following PCR amplifications. The deduced amino-acid sequence showed high similarity with SdhC from other organisms. Comparison of *AaSdhC* full sequence from sensitive and resistant isolates revealed that a highly conserved histidine residue (codon CAC in sensitive isolates) was converted to arginine (codon CGC, in resistant isolates, type III mutants) at position 134. A PCR-RFLP assay in which amplified gene fragments were digested with *Aci*I was successfully employed for the diagnosis of these type III mutants. This molecular assay will greatly improve the monitoring of the boscalid resistant *Alternaria* spp. of pistachio. To our knowledge, this is the first report of fungal field boscalid-resistant isolates carrying mutation in the SdhC subunit.

MOI-106: A new alternative for controlling fungal plant pathogens in ornamentals and edible crops

C. AVILA-ADAME (1), E. Tan (1), B. Campbell (1), H. Huang (1), L. Fernandez (1), M. Koivunen (1), P. Marrone (1)
(1) Marrone Organic Innovations, Inc, Davis, CA, USA
Phytopathology 98:S16

MOI-106 is an aqueous formulation of an ethanol extract derived from the plant giant knotweed, *Reynoutria sachalinensis*. This biopesticide is highly active against several powdery mildew species and *Botrytis* spp. Treatments with this compound should be applied before infection occurs due to the fungicide's prophylactic properties. The mode of action of *R. sachalinensis* extract has been described as a plant defense inducer, and two anthraquinones, Emodin and Physcion, are likely associated with this activity. The efficacy of MOI-106 to control fungal diseases is dose dependent as determined by a bioassay analysis using cucumber plants infected with powdery mildew. In three experiments, two-week old cucumber plants were treated with MOI-106 at concentrations ranging from 0.008 to 1.0% (v/v). HPLC analysis indicated that the formulated extract contained 385.6 and 306.8 µg/ml of Emodin and Physcion, respectively. Four hours after treatment, plants were inoculated with powdery mildew conidia in a water-suspension containing 21 to 64 × 10^4 conidia per ml. The ED_{50} values estimated 9 to 11 days after inoculation were 0.38 µg/ml for Emodin and 0.31 µg/ml for Physcion. Emodin and Physion fractions were also tested independently and both compounds alone provided significant efficacy in controlling cucumber powdery mildew.

Chemotaxic effects of endophyte-infected tall fescue root extracts against *Pratylenchus scribneri*

A. A. BACETTY (1), M. E. Snook (2), C. W. Bacon (2)
(1) The University of Georgia, Department of Plant Pathology, Athens, GA; (2) Toxicology and Mycotoxin Research Unit, USDA, ARS, Russell Research Center, Athens, GA
Phytopathology 98:S16

Protection against herbivores and insects in the *Neotyphodium*-tall fescue grass association is provided by toxic secondary metabolites. Root exudates in this relationship contains both hydrophobic and hydrophilic substances released into the soil that are either stimulatory, inhibitory, or inactive to above and below ground pests. Through previous *in vitro* nematode bioassay experiments it was revealed that phenolic compounds produced in this fungal-grass association exhibited adverse effects to *Pratylenchus scribneri*. Current research is presented that has identified several groups of potential allelochemicals from endophyte-infected grasses, plant and fungus produced. The results indicate that there is a behavioral response indicative of a chemosensitivity of the nematode *P. scribneri* when subjected to a class of phenolic compounds suggesting a means of dissecting plant host response from endophyte induced responses. The behavioral changes of *P. scribneri* in response to this and other allelochemicals predicts that studies of taxis and kinesis of this and other nematodes species may form the basis for distinguishing and identifying the basic grass response from the fungus induce response.

Interaction of fusaric acid and maize seedling lesion development and reduction by isolates of *Bacillus mojavensis*

C. W. BACON (1), D. M. Hinton (1)
(1) USDA, ARS, Russell Research Center, Athens, GA
Phytopathology 98:S16

Due to autoinfection and alloinfection, maize is parasitized by *Fusarium verticillioides* and subject to contamination from its mycotoxins, the fumonisins. Attempts at controlling both the disease and mycotoxins resulting from infection are desirable since in some cultivars infections do not always lead to a disease unless maize is cultured under unfavorable conditions, although the mycotoxins are always produced. The fungus also produces fusaric acid which acts both as a phytotoxin and an antibiotic to microorganisms, especially bacteria. The primary objective of this work was to determine the efficacy of the biocontrol endophytic bacterium *Bacillus mojavensis* and its fusaric acid tolerant mutants in the control of lesion development indicative of maize seedling blight by *F. verticillioides* and the

interaction of fusaric acid with lesion development. Three strains of this fungus that did or did not produce fusaric acid were targets. Lesions were measured in two cultivars of 41-day-old maize stems consisting of treatment groups inoculated with and without mutants and wild type strains of bacteria and fungi. The lesion test was also used on 16-day-old maize plants inoculated with several stains of *B. mojavensis* in an effort to determine stains of this bacterium that might provide more protection. The results suggest that fusaric acid does interact in planta for the production of lesions but both wild type and mutant bacteria that are tolerant of this phytotoxin are competent in reducing lesions produced by virulent and nonvirulent strains of *F. verticillioides*, suggesting that fusaric acid may not be produced during this early infection process used in this study.

In planta expression of a soluble recombinant form of the GN glycoprotein (GN-S) of *Tomato spotted wilt virus* (TSWV) and assessment of its interaction with western flower thrips (WFT) gut

I. E. BADILLO-VARGAS (1)
(1) University of Wisconsin - Madison
Phytopathology 98:S17

Tomato spotted wilt virus (TSWV) infects over 1,050 different plant species in more than 70 distinct botanical families and is responsible for substantial losses in fruits, vegetables, ornamentals, and fiber crops. Its wide distribution throughout tropical, subtropical, and temperate zones combined with its specific interaction and dependence on thrips for transmission make this virus highly important in agricultural settings. TSWV is the prototype member of the *Tospovirus* genus within the *Bunyaviridae* family and the western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is its most efficient vector. TSWV is transmitted in a persistent and propagative manner only if it is acquired during larval stages. However, it is retained during molting and lasts until thrips adulthood. The significant problems associated with this widespread and complex pathosystem are likely to be exacerbated by climate changes. The viral envelope glycoproteins, G_N and G_C, which are critical for infection of thrips but are not required for the initial infection of plants, are presumed to be the viral determinants that mediate the specific interaction between TSWV and WFT. A soluble recombinant form of the G_N glycoprotein (G_N-S) and a G_N-S-GFP fusion peptide have been expressed in *Nicotiana benthamiana* plants using *Agro*-infiltration. We will confirm transgene transcription in transformed plant tissue using RT-PCR. Western blot analysis will be used to detect the biosynthesis of G_N-S and G_N-S-GFP *in planta*. Finally, we will assess the specific binding of this viral glycoprotein surrogate to thrips midgut using *Agro*-inoculated plants expressing G_N-S and G_N-S-GFP in an *in planta* binding assay. Plants expressing only GFP will be used as negative control to prove the specificity of the interaction between G_N-S and WFT midgut. A positive interaction of G_N-S within thrips gut could lead to the development of efficient antiviral strategies to control TSWV.

***Trichoderma* species colonize *Theobroma cacao* trichomes internally**

B. A. BAILEY (2), M. D. Strem (2), D. F. Wood (1)
(1) USDA/ARS/WRRC, Albany, CA, USA; (2) USDA/ARS/SPCL, Beltsville, MD, USA
Phytopathology 98:S17

Trichoderma species have been extensively studied for their capabilities to control plant diseases. With continued study has come the understanding that *Trichoderma* species establish complex interactions with plants. *Trichoderma* species used in biological control of plant disease are usually considered soil organisms that colonize plant roots and some can form a symbiotic relationship. Recent studies demonstrated that *Trichoderma* species are also capable of colonizing the above ground portions of *Theobroma cacao* (cacao) in what has been characterized as an endophytic relationship. *Trichoderma* species can be re-isolated from cacao stem tissue, including the bark and xylem, the apical meristem, and to a lesser degree from leaves. Hyphae were observed emerging from the glandular trichomes on surface sterilized stems from cacao seedlings that had been inoculated with one of four *Trichoderma* species (*T. ovalisporum*, *T. hamatum*, *T. koningiopsis*, or *T. harzianum*). Trichomes were isolated from surface sterilized stems and plated on corn meal dextrose agar media. Fungal hyphae were observed under the microscope emerging from the isolated trichomes after 6 hrs. Repeated single trichome/hyphae isolations verified that the emerging hyphae were the *Trichoderma* species with which the cacao seedlings were inoculated. SEM analysis of *Trichoderma* colonized stems showed a preference for surface colonization of glandular trichomes verses non-glandular trichomes. The *Trichoderma* isolates colonized the glandular trichome tips and some isolates formed swellings that resemble appresoria. Four *Trichoderma* species are able to enter the glandular trichomes during the colonization of cacao stems where they survived surface sterilization and could be re-isolated. The penetration of cacao trichomes may provide the entry point for the *Trichoderma* species into the cacao stem allowing systemic colonization of the tissue.

Mass production of Alternaria alternata isolates: Bioherbicide agents for Rumex dentatus and Chenopodium album

R. BAJWA (1)
(1) University of the Punjab, Lahore, Pakistan
Phytopathology 98:S17

Laboratory evaluation was carried out of locally available wheat straw, rice straw and bagasse as substrates supplemented with chickpea flour and molasses for mass culturing of *Alternaria alternata* (Fr.) Keissler. isolates, potential bioherbicides for *Rumex dentatus* L. and *Chenopodium album* L. Among these three substrates wheat straw with chickpea flour as supplement produced 213 conidia/10 ml in case of strain R (strain of *A. alternata* isolated from *R. dentatus*) and 203 conidia/10 ml in strain C (strain of *A. alternata* isolated from *C. album*) after 14 days with 12 hours photoperiod at 25°C–30°C. The viability test of the *A. alternata* conidia @ 107 conidia/ml and 109 conidia/ml for strain R and strain C respectively mass produced on selected suitable substrate against 4–5 leaf stage of *R. dentatus* and 10–15 leaf stage of *C. album* revealed 100% mortality of the target weeds at 100% humidity for 24 h and at 25°C–30°C.

Evaluation of multiple-locus variable number tandem repeat analysis for typing of *Pseudomonas syringae*

C. M. BAKER (1), C. L. Bender (1), U. K. Melcher (1), J. A. Fletcher (1)
(1) Oklahoma State University, Stillwater, OK, USA
Phytopathology 98:S17

Pseudomonas syringae pv. *tomato* strain DC3000 (*Pst* DC3000) is a gram-negative plant pathogenic bacterium that causes bacterial speck, an economically important disease of tomato. *P. syringae* shares survival and virulence mechanisms with pathogens that are currently on the USDA APHIS select agent list, but is a model pathogen that can be handled without biocontainment facilities. Studies with other bacterial species demonstrated that variable number tandem repeat (VNTR) loci, which are found throughout bacterial genomes, were useful for rapid and reliable strain typing by multiple-locus VNTR analysis. In the current study, the genome of *Pst* DC3000 was analyzed, and oligonucelotide primers were designed to amplify 34 VNTR loci. The primers were screened using seven representative strains of pathovar tomato, and a subset of five primer pairs yielding polymorphic products was identified for use in strain typing. When used to type a collection of 60 diverse pathovar tomato strains, these five primer pairs discriminated among some strains of pathovar tomato. This molecular typing tool can be applied to other phytopathogenic bacteria, and may be useful for tracing bacterial outbreaks and dissemination, as well as for studying bacterial population structure and diversity. Research on strain typing and identification techniques benefit society through improved safety and security of our food supply.

Plant diversity effects on microbial diversity and pathogen suppression

M. G. BAKKER (1)
(1) University of Minnesota
Phytopathology 98:S17

There is little understanding of the ecological and evolutionary factors that promote pathogen suppressive microbial communities. We hypothesize that high density and diversity microbial communities create a selective environment that gives rise to pathogen suppression. Because of the influence of plant inputs on rhizosphere microbial dynamics, reduced plant diversity is predicted to reduce microbial diversity and pathogen suppressive activity. Using experimental plots at the Land Institute (Salina, KS), we analyzed the pathogen suppressive ability and phylogenetic and phenotypic diversity of soil Actinomycete communities associated with high and low diversity plant communities. Soil cores were drawn from three replicate plots of virgin prairie and never-plowed annual monocultures that had been converted directly from prairie to no-till agriculture. One hundred and fifty bacterial isolates showing typical Actinomycete morphology were collected from each soil. Partial 16S rDNA sequence was obtained for each isolate, and each isolate was tested for inhibition of four different plant pathogens. The phylogenetic diversity and pathogen suppressive ability of the Actinomycete communities correlate with plant diversity. The frequency, intensity, and diversity of pathogen-inhibitory activities are distinct characteristics of a given microbial community, and all are likely to be critical for effective and durable pathogen control.

Dynamics of *Sclerotinia homoeocarpa* populations to curative applications of specific-site fungicides

J. BAN (2), Y. Jo (1), P. Koch (3), G. Jung (2)
(1) Department of Plant Pathology & Microbiology, 120 Peterson Building, College Station, TX 77843; (2) University of Massachusetts, 206 French Hall, Amherst, MA 01003; (3) University of Wisconsin, Madison, WI 53706
Phytopathology 98:S17

Dollar spot, caused by *Sclerotinia homoeocarp*a, is one of the most intensively managed turfgrass diseases in North America. Due to repeated

fungicide applications, resistance has become a major concern for golf course superintendents. The objective of this research was to study the effect of curative, repeated applications of specific-site fungicides (thiophanate-methyl and propiconazole) on dynamics of dollar spot populations with different levels of fungicide sensitivities to these fungicides. Thiophanate-methyl applications were withheld for one growing season and the resistant populations were monitored. Field plots were established on the fairway and putting green turfgrass at the O.J. Noer Turfgrass Research and Education Facility at Verona, WI and on the fairway at the Joseph Troll Turf Research Center at South Deerfield, MA. The dollar spot population in MA was sensitive to thiophanate-methyl and propiconazole but the population found in WI showed some levels of insensitivities to both fungicides. Resistant populations were rapidly selected up to 100% by curative, repeated applications of thiophanate-methyl regardless of initial resistance levels in the populations. The thiophanate-methyl resistant populations in the absence of the same fungicide for one growing season were decreased at the MA site but no change in resistance was observed at the WI site. With propiconazole, the MA population was never exposed previously to this fungicide and seemed to respond to the repeated applications in a slow manner of fungicide resistance development. In the WI populations, however, the fairway population shifted to propiconazole insensitivity faster than the green population. Our results suggest that dynamics and persistence of resistant populations depend on initial populations as well as different specific-site fungicides.

Effect of water potentials on sclerotial germination of *Macrophomina phaseolina* and predisposition of sorghum to charcoal rot

Z. BANIHASHEMI (1), A. Goudarzi (2)
(1) Shiraz University; (2) Student
Phytopathology 98:S18

Macrophomina phaseolina is a widespread soil borne plant pathogen in Iran infecting various agricultural crops especially under environmental stresses. Effect of water potential on sclerotial germination of a melon isolate of *M. phaseolina* and disease development in sorghum was investigated. For sclerotial germination, six levels (0, –0.3, –0.6, 0.9, –1.2, –1.5 MPa) of osmotic (NaCl) and matric (PG6000) potentials were used. Maximum germination occurred at –1.2 and –0.6 MPa at matric and osmotic potentials respectively. In a greenhouse study, effect of salinity, water stress and their interactions on the predisposition of sorghum to *M. phaseolina* was also investigated. Four levels of salinity (0, 1400, 2100, 2800 mg NaCl/Kg soil), three irrigation intervals (3, 7, 10 days) and their interactions were compared. Six-week-old sorghum seedlings was transferred into infested and non-infested soil and gradually exposed to salt stress. Increasing irrigation intervals increased root colonization in infested soil and reduced shoot and root dry weight and salt injury in infested and non-infested soils. Root colonization increased with increasing salinity level up to 1400 mg NaCl/Kg soil. Salinity and *M. phaseolina* interaction increased the concentration of Na and Cl compared to salinity stress *per se* but negatively correlated with increasing irrigation intervals.

University of Florida Plant Medicine Program Clinical Trials

A. C. BARBAGLIA (3), T. C. Durham (3), K. H. Cho (3), S. J. Glucksman (3), E. B. Rivera Del Cid (1), C. R. Semer (3), R. J. McGovern (2)
(1) Escuela Agricultura PanAmericana (Zamorano), Tegucigalpa, Honduras; (2) University of Florida-IFAS, Dept. of Plant Pathology and Plant Medicine Program, 1453 Fifield Hall, Gainesville, FL 32611; (3) University of Florida-IFAS, Plant Medicine Program, Clinical Trials, 1453 Fifield Hall, Gainesville, FL 32611
Phytopathology 98:S18

We agree with the recent statement made by Cooke et al. that “All forms of professional education share the goal of readying students for accomplished and responsible practice in service to others”. Established in 2005, the Clinical Trials has become an integral part of the University of Florida Plant Medicine Program. The objective of the Clinical Trials is to provide “hands-on” training in the design and execution of laboratory, greenhouse and field experiments in plant health management, analysis and interpretation of data and presentation of research results. A new course, Research Methods in Plant Health Management, has been developed to provide graduate students with the basic statistical background and practical experience needed to conduct experiments. Model systems for instruction include management of weeds, arthropods and pathogens using both conventional and alternative practices. Experiments conducted in the Clinical Trials are supported by a variety of corporate entities. We believe that the Clinical Trials prepares future plant doctors to effectively carry out research in plant health management as part of their employment or in service to their clientele, and to accurately interpret and apply relevant scientific literature.

Patterns of *Phakopsora pachyrhizi* spore deposition detected in North America rain and their use to calibrate IAMS soybean rust forecasts in 2007

C. W. BARNES (2), L. J. Szabo (2), S. A. Isard (5), A. Ariatti (5), A. U. Tenuta (4), S. Hambleton (1), R. Tropiano (1), V. C. Bowersox (3), R. Claybrooke (3), C. Lehmann (3)
(1) Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; (2) CDL, USDA-ARS, University of Minnesota, St. Paul, MN, USA; (3) NADP, Illinois State Water Survey, Champaign, IL, USA; (4) Ontario Ministry of Agriculture, Food and Rural Affairs, Ridgetown, Ontario, Canada; (5) Penn State University, University Park, PA, USA
Phytopathology 98:S18

In 2007, rain was assayed for *Phakopsora pachyrhizi* spores (the causal agent of Asian soybean rust) at 75 National Atmospheric Deposition Program sites in the U.S. and at 12 sites in Canada using two types of rain collectors. A nested real-time PCR assay was used to detect *P. pachyrhizi* DNA in the filtered residue. This year, *P. pachyrhizi* spores were detected 90 times in the U.S. and 29 times in Canada. The average detection percentage from U.S. sites was 8.5% of rain samples, with a peak of 27% in mid August. The number of spores per collection was estimated using models generated by assaying a known number of *P. pachyrhizi* spores. Estimates ranged from a single spore/m^2 to over 500. *P. pachyrhizi* spores were generally detected 3–5 weeks prior to first disease reports in a state, matching IAMS disease forecasting model estimates. Spore deposition results from the U.S. and Canada were used to further calibrate the IAMS model. Two time intervals were analyzed because they showed the clearest indication of long-distance transport of spores; July 9–18 and Aug 13–28, 2007. During the mid July interval, 9 rain samples were positive for *P. pachyrhizi* at 9 different U.S. sites, while there were 11 positive rain samples at 8 Canadian sites. The positive sites tended to cluster around Lake Erie, with estimates of 186 spores/m^2 in central Ohio. During the August transport event, 29 U.S. and 9 Canadian sites were positive for *P. pachyrhizi*. However, the focus of deposition was further west in the central plains north to Manitoba and Saskatchewan, with the highest level of spore deposition in Iowa at 243 spores/m^2. This second deposition event correlates with the finding of Asian soybean rust disease in this region in late September and October.

Proteomic analysis of potato late blight resistance mediated by the *RB* resistance gene

G. Barrett-Wilt (2), E. Huttlin (1), A. Harms (2), M. Sussman (1), D. HALTERMAN (3)
(1) UW Biotechnology Center Mass Spectrometry/Proteomics, Department of Biochemistry, University of Wisconsin-Madison, USA; (2) UW Biotechnology Center Mass Spectrometry/Proteomics, University of Wisconsin-Madison, USA; (3) Vegetable Crops Research Unit, USDA-ARS, University of Wisconsin-Madison, USA
Phytopathology 98:S18

Late blight, caused by the oomycete pathogen, *Phytophthora infestans*, is a devastating disease of potatoes and tomatoes. A gene *RB*, cloned from the Mexican diploid potato species *Solanum bulbocastanum*, confers robust resistance to potato late blight. *RB* encodes a CC-NBARC-LRR (coiled coil, nucleotide binding site, leucine-rich repeat) resistance protein. RB is involved in perception of *P. infestans*, through recognition of the effector ipiO, and signaling to elicit resistance within the plant cell cytoplasm. By studying the proteins involved in the resistance response, we hope to better understand the disease resistance mechanisms mediated by *RB*. We are using quantitative analysis of protein abundance using nitrogen15 (N15) labeling of all plant proteins, followed by analysis using multidimensional protein identification technology (MudPIT). In all experiments, we are comparing proteins isolated from a late blight resistant transgenic potato plant SP951, containing a single copy of *RB*, with those from the susceptible control Katadhin (Solanum tuberosum cv. Katadhin) at 0, 24, and 72h post-inoculation with *P. infestans*.

Influence of quinic acid catabolism on the production of the plant growth regulator phenylacetic acid by *Rhizoctonia solani* AG-3

F. E. BARTZ (3), D. A. Danehower (2), S. Tavantzis (1), M. A. Cubeta (3)
(1) Department of Biological Sciences, The University of Maine, Orono, ME, USA; (2) Department of Crop Science, North Carolina State University, Raleigh, NC, USA; (3) Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA
Phytopathology 98:S18

The soil fungus *Rhizoctonia solani* AG-3 is a pathogen that causes seedling damping-off and canker diseases of plants in the family Solanaceae. The parasitism and infection process of *R. solani* is associated with production of the plant growth regulator phenylacetic acid (PAA). The biosynthetic pathway that leads to the production of PAA shares two intermediates with the substrate inducible quinic acid (QA) carbon catabolism pathway. Tavantzis and colleagues have shown that utilization of QA by *R. solani* AG-3 competes

for these intermediates and reduces the severity of canker disease on potato. In this study, experiments were conducted to quantify the effects of QA on mycelial growth and PAA production of *R. solani* AG-3. Biomass was quantified by measuring the mycelial dry weight of each isolate after 3 wk incubation in Vogel's minimal medium amended with concentrations of QA ranging from 0–250 mM. All isolates utilized QA as a carbohydrate source, with more biomass produced in the QA treatments than in the control. Isolates varied in their growth rates and in the shape of their growth curve in response to increasing concentrations of QA. Gas chromatography was used to quantify PAA and its hydroxy and methoxy derivatives produced in the 0 and 25 mM QA treatments for each isolate. Isolates varied in the quantity and type of PAA produced. There was a significant reduction in the production of PAA and or its derivatives for all isolates when grown with QA.

Relationship between huanglongbing severity and reduction of yield in 'Valência' orange

R. B. Bassanezi (1), L. AMORIM (2), L. H. Montesino (1), M. C. Gasparoto (2), A. Bergamin-Filho (2)
(1) Fundecitrus, Araraquara, SP, Brazil; (2) USP-ESALQ, Piracicaba, SP, Brazil
Phytopathology 98:S19

Huanglongbing (HLB) is the most severe disease of citrus. Since 2004, when disease was officially reported in Brazil, more than 2 million trees were eradicated from commercial citrus groves. Yield of affected trees is reduced by the early drop of fruits and by low quality of affected fruit, which are small and lopsided. Juice from diseased fruit is very acid and has reduced brix. The yield reduction can reach 100% depending on disease severity. The purpose of this work was to quantify the yield reduction on trees of cv. Valência affected by HLB. One hundred trees with different levels of disease severity (% of affected canopy area) were selected in five plots belonging to three different groves. Disease severity was visually estimated and yield was measured in each of the 500 selected trees. Total number of fruit, number of diseased fruit and weigh of health and diseased fruit were measured at harvest. The reduction of yield in diseased trees, compared to the average yield of health trees was calculated. Total number of fruit and the number of health fruit were related to disease severity by the model $y = a\exp(-bx)$ where y is the yield component and x, disease severity. Rate parameter b was similar for all plots from different groves (close to 0.018 for both reduction in fruit number and reduction in fruit weight). Intercept parameter a was similar in plots from each grove but showed significant differences between groves. Reduction of yield (proportion of number of fruit or of weight of fruit) was related to disease severity by the monomolecular equation. Equations were similar for all plots. Disease severity was related to reduction of yield by $y = 1\text{-}1.05\exp(-0.016x)$ and to reduction of fruit weight by $y = 1\text{-}0.98\exp(-0.05x)$.

Transgene expression in the basidiomycete root pathogen *Armillaria mellea*

K. BAUMGARTNER (2), A. Bailey (1), G. D. Foster (1), S. Kilaru (1)
(1) School of Biological Sciences, University of Bristol, England; (2) USDA-ARS, Davis, CA, USA
Phytopathology 98:S19

Toward development of a genetic transformation system for *Armillaria mellea*, we used particle bombardment to identify promoters for driving transgene expression. The plasmid tested was pYES-hph-004iGFP, on which the green fluorescence protein gene, *gfp*, is linked to the *Agaricus bisporus gpdII* promoter and the *Phanerochaete chrysosporium mnp1* terminator. Malt extract agar was inoculated with *A. mellea* (incubated at 25C, 24 d). YMGT agar (0.4% yeast extract, 1% malt extract, 1% agar, 0.01% tryptophan, 0.04% Dglucose) was inoculated with *Coprinopsis cinerea* (incubated at 37C, 5 d; positive control). Gene gun cartridges were prepared the day of use (6.25 mg 0.6-mm gold, 0.05M spermidine, 0.05 mg/ml PVP, 100 mg DNA per 25 in. tubing). Cartridges prepared with plasmid DNA or no DNA (negative control) were applied at 150 and 200 psi to each of 21 *A. mellea* and *C. cinerea* colonies (Helios Gene Gun; Bio-Rad, Hercules, CA). Petri plates without lids were positioned 4 cm from the cartridge, with no screen between the barrel and agar surface. Each culture received two shots per cartridge. After 2 d, cultures were examined under fluorescent light on a compound microscope (Leica MZFL111; 100X, 450-490 nm excitation filters, 510 nm dichroic filter, 515 nm emission filter). The presence of a green spot among *A. mellea* hyphae bombarded with pYES-hph-004iGFP-coated particles verified that a single cell was penetrated by a particle, and that *A. mellea* is capable of translating *gfp* under control of the *gpdII* promoter. This is the first instance of transgene expression in *Armillaria*. Expression was visible for at least one week, but subcultures did not express gfp after one month; expression was transient. Nonetheless, this is an achievement because plasmids for both *Agrobacterium*-mediated and protoplast/PEG mediated transformation that have been shown to give the highest transformation success among other Basidiomycetes with the *gpdII* promoter.

Diversity and distribution of *Ceratobasidium* and *Thanatephorus*: What orchid mycorrhizal fungi can tell us

P. BAYMAN (2), A. Porras-Alfaro (1), J. Otero (3)
(1) Biology Department, University of New Mexico; (2) Departamento de Biología, Universidad de Puerto Rico - Río Piedras; (3) Departamento de Ciencias Biológicas, Universidad Nacional de Colombia-Palmira
Phytopathology 98:S19

Ceratobasidium and *Thanatephorus* include important plant pathogens, but they also include mycorrhizal fungi of orchids. The lifestyles, distribution and phylogenetic affinities of some of these fungi are unknown, especially in the tropics. Fungi were isolated from pelotons in orchid roots and identified by sequencing the nuclear ribosomal ITS region from pure cultures and direct amplifications from colonized root tissue. Several novel results were obtained: 1) Most orchid-associated fungi did not appear to belong to currently described species. 2) A single ITS type of a recently discovered *Ceratobasidium* clade was associated with the orchid *Ionopsis utricularioides* and unexpectedly widespread throughout Central America and the Caribbean. 3) *Ceratobasidium* was significantly more common in *Vanilla* roots on tree bark than in *Vanilla* roots in soil. This was surprising given that *Ceratobasidium* is rarely reported from tree bark. 4) Some strains of *Thanatephorus* showed extremely limited growth in culture media. Growth limitations of *Thanatephorus* strains have not been reported previously. The phylogeny of the *Ceratobasidium/Thanatephorus* group is still unresolved, and the addition of mycorrhizal members may help clarify relationships.

Efficacy of biopesticides and fungicides against pre- and post-emergence damping-off of vegetable seedlings by *Pythium aphanidermatum*

F. BAYSAL GUREL (1), S. A. Miller (1)
(1) Department of Plant Pathology, The Ohio State University-OARDC, 1680 Madison Ave., Wooster, OH, USA
Phytopathology 98:S19

Damping-off, caused by *Pythium aphanidermatum*, can result in significant losses in transplant production in protected environments. Experiments were performed in a greenhouse to determine the efficacy of biopesticides and fungicides against *Pythium* damping-off in cabbage and pepper. Treatments applied were the biofumigant QRD 300 (*Muscodor albus*), composted cow manure (10%), Omega Grow and Omega Grow Plus (fish proteins), Seacide (fish emulsion), the biocontrol agents Serenade ASO, *Trichoderma hamatum* 382 (T382), *Pseudomonas fluorescens*-Wayne 1R and Mg1 A2R, Mycostop, Prestop, and the fungicides Phosphonate, Ranman and Thiram. Incorporation of composted cow manure or T382 into the planting mix, drench application of Ranman, and Thiram seed treatment increased the percentage of healthy seedlings of both cabbage and pepper. Omega Grow reduced damping-off in pepper but was phytotoxic to cabbage seed. QRD 300 (3.5 g/L planting mix) increased the percentage of healthy cabbage seedlings compared to the untreated controls. The higher rates of Serenade ASO (1% and 2%) were significantly better than the low rate (0.5%) in increasing the percentage of healthy cabbage but not pepper seedlings. Phosphonate, *P. fluorescens*-Wayne 1R and Mg1 A2R, and Mycostop increased the percentage of healthy seedlings in cabbage compared to the untreated controls, but neither Seacide, Phosphonate, Prestop, *P. fluorescens*-Wayne 1R and Mg1 A2R nor Mycostop increased the percentage of healthy pepper seedlings. Cabbage and pepper seedlings produced in composted cow manure mix were taller than the inoculated, untreated controls.

Suppression of *Rhizoctonia solani* in soils under different transitional organic management practices

F. BAYSAL GUREL (2), M. Kleinhenz (1), J. Cardina (1), B. McSpadden Gardener (2), S. A. Miller (2)
(1) Department of Horticulture and Crop Sciences, The Ohio State University-OARDC, 1680 Madison Ave., Wooster, OH, USA; (2) Department of Plant Pathology, The Ohio State University-OARDC, 1680 Madison Ave, Wooster, OH, USA
Phytopathology 98:S19

The influence of transitional organic soil management practices on suppression of *Rhizoctonia solani* was studied using radish as an indicator. Treatments included clean fallow, mixed hay, open-field vegetables, and high tunnel vegetables over a 4-year transition period. Compost (18.6 T/ha d.w.) was applied to one-half of each treatment plot. The field experiment was conducted under high tunnels in fall, 2006. Compost amendment significantly reduced the incidence of Rhizoctonia root and hypocotyl rot in naturally infested soil by approximately 30% and increased total yield by approximately 50% and marketable yield by approximately 15%. Soil samples were removed from all treatments in the field site in spring, 2007, and assayed for damping-off and root and hypocotyl rot of radish in growth chambers in the presence and absence of added *R. solani* inoculum. Compost amendments significantly reduced the incidence and severity of Rhizoctonia damping-off and root and hypocotyl rot, in both the presence and absence of added inoculum.

Interestingly, disease suppressiveness was greatest in soil collected from the mixed hay plots, regardless of compost amendment or pathogen pressure. Nonetheless, plant height, leaf number and/or fresh weight of the surviving radishes were greater in the compost-amended than non-amended soils regardless of the transitional treatments used.

Oxalic acid production by *Sclerotinia homoeocarpa*: The causal agent of dollar spot

R. A. BEAULIEU (2), A. Martínez Medina (1), M. J. Boehm (2)
(1) CEBAS-CSIC, Murcia, Spain; (2) Dept. Plant Pathology, The Ohio State University, Columbus, OH, USA
Phytopathology 98:S20

Fungi in the genus *Sclerotinia* include some of the most devastating pathogens known. Dollar spot, caused by *S. homoeocarpa*, is the most prevalent and sprayed for disease of golf course turf. The production of oxalic acid and pectolytic cell wall-degrading enzymes by species within this genus is well documented. A series of laboratory-based assays were used to determine whether *S. homoeocarpa* produces oxalic acid. Potato dextrose agar (PDA) adjusted to pH 4 and pH 6, with and without bromophenol blue (Bb), was used to assess the growth of and acid production by *S. homoeocarpa* between 5–35 C. When added to PDA, Bb only slightly hindered the growth of *S. homoeocarpa*. Acid production by *S. homoeocapra* on PDA + Bb occurred between 15–30 C at both pHs, but was first observed on media adjusted to pH 6. Maximum acid production occurred between 20–30 C. Acid production by *S. homoeocarpa* was also observed when grown in potato dextrose broth (PDB) at 25 C. High Performance Liquid Chromatography analysis of spent culture broth collected from *S. homoeocrapa* inoculated PDB revealed the presence of oxalic acid.

How different are the two model Fusarium graminearum strains PH-1 and Gz3639 from one another?

S. BEC (1), L. J. Vaillancourt (1), D. Van Sanford (2)
(1) Department of Plant Pathology, University of Kentucky, Lexington, KY, USA; (2) Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA
Phytopathology 98:S20

Fusarium graminearum is the primary causal agent of the head blight disease that severely affects small grains. Two *F. graminearum* strains have been used as models by different laboratories studying the head blight disease. PH-1 (NRRL 31084) has been used most frequently for research focused on developmental biology, fertility, and genomics, while Gz3639 is the standard strain that has been used for most mycotoxin biosynthesis research. The genome sequences of both strains have recently been made available to the research community. Although there is quite a lot of anecdotal evidence about how these strains differ, no direct side-by-side comparisons of the two strains have been reported. In spite of a high level of genome sequence similarity we found that the two strains differ significantly in colony characteristics, growth rate, asexual and sexual spore production, fertility, and levels of mycotoxin production. We compared the behavior of the two strains on various liquid and solid media. The pathogenicity and aggressiveness were compared by point inoculation during early anthesis of wheat heads carrying different type of resistance to Fusarium head blight. The two strains produce similar disease symptoms when inoculum levels are high, but Gz3639 is less aggressive at lower levels. To more closely examine the genetic regulation of traits impacting the fertility, pathogenicity, and aggressiveness of each strain we made a cross between PH-1 and Gz3639 and we are in the process of selecting the progeny and analyzing co-segregation patterns among traits.

Widespread occurrence and molecular characterization of barley dwarf geminivirus in Iran

A. Behjatnia (1), A. Afsharifar (1), V. Tahan (1), V. Amid Motlagh (1), O. Eini Gandomani (2), A. Niazi (1), K. IZADPANAH (1)
(1) Shiraz University; (2) Zanjan University
Phytopathology 98:S20

Yellows diseases cause considerable losses in many wheat and barley fields throughout Iran. Association of barley yellow dwarf viruses with such diseases has been reported previously. However, there have been many cases in which no barley yellow dwarf viruses have identified. In the present paper, we report widespread association of Iranian isolate of barley dwarf geminivirus (BDV-Ir) with yellows diseases of cereal crops in this country. BDV-Ir was identified in many yellow-affected samples of barley and wheat from northern, northwestern, northeastern, central and southern Iran using PCR with specific primers. The full length sequence of theBDV-Ir genome was determined. It showed 90–91% similarity with the complete genome of BDV isolates from Germany at nucleotide level. Using dot blot hybridization with full genome as probe, BDV-Ir was detected in 35 of 156 yellows affected samples. It is concluded that BDV-Ir is a major component of yellows complex in cereal fields in Iran. This study was supported by funds from Centers of Excellence and TWAS-IC.

A semi-selective medium for the isolation of copper and streptomycin resistant strains of *Xanthomonas citri* ssp. *citri* from plant material

F. BEHLAU (1), J. B. Jones (1), J. H. Graham (2)
(1) University of Florida, Gainesville, FL 32611; (2) University of Florida, Lake Alfred, FL 33850
Phytopathology 98:S20

After eradication efforts were halted, attention has focused on strategies to control citrus canker (*Xanthomonas citri* ssp. *citri* - Xcc), including use of bactericides such as copper (Cu) and streptomycin (Str). A concern in using these chemicals is that widespread use may lead to development of resistant strains which are difficult to detect within the total bacterial population. A semi-selective medium, MGY-KCC, was developed to recover Cu and Str resistant strains of Xcc from plant material. MGY agar (mannitol glutamate yeast agar, mannitol 10 g L^{-1}, L-glutamic acid 2 g L^{-1}, KH_2PO_4 0.5 g L^{-1}, NaCl 0.2 g L^{-1}, $MgSO_4.7H_2O$ g L^{-1}, yeast extract 1.0 g L^{-1}, agar 15 g L^{-1}), a standard medium utilized to assess Cu resistance, was used as the basic medium. The antibiotics, kasugamycin (12 mg L^{-1}), cephalexin (12 mg L^{-1}), and cycloheximide (40 mg L^{-1}), and the salt KBr were added to MGY to suppress contaminants and enhance xanthomonadin pigment, respectively. Cu and Str resistant bacteria present in symptomatic leaf washings were assessed by amending the medium with CuSO4 (50 mg L^{-1}) or streptomycin sulfate (25 mg L^{-1}). Xcc strains resistant to Cu or Str were efficiently recovered on MGY-KCC amended with Cu or Str, respectively, from washings of symptomatic grapefruit leaves. MGY-KCC significantly reduced the presence of contaminants and will be a useful tool for screening the presence of Cu and Str resistant strains in citrus canker lesions and in the phyllosphere.

Efficiency of endoparasitic nematode extraction from corn roots

J. L. BEHN (1), A. E. MacGuidwin (2), T. A. Jackson (1)
(1) University of Nebraska-Lincoln; (2) University of Wisconsin-Madison
Phytopathology 98:S20

The diversity of nematodes and prevalence of endoparasites in corn production areas necessitates the extraction of nematodes from plant roots in addition to soil to accurately identify them and estimate their potential to cause crop injury. There are numerous extraction methods yet no standardized protocol is in use by nematology labs. Two methods, the Baermann funnel and the shaker, were evaluated in 2007 for nematode extraction from different corn root types. Corn root and soil samples were collected on 5 Sep and 9 Oct; they were then grouped by root type (seminal, branch, and anchor) and extracted by the two methods. Nematodes were also extracted via the Baermann funnel from the root fragments that were captured from the soil samples. There were significant ($P < 0.01$) 2-way interactions between extraction method and both corn root type and sampling date. The extraction rate for Pratylenchus spp. (root-lesion nematode) was highest with the Baermann funnel for all root types. The root fragments found in soil yielded the highest population densities of Pratylenchus spp., averaging 420% more nematodes than those associated with other root types. These preliminary results indicate that the Baermann funnel technique is more effective than continuous shaking in extracting *Pratylenchus* spp. from corn roots of all types.

Landscape-scale biogeography of *Sclerotinia homoeocarpa* causing turfgrass dollar spot disease across New Jersey and the New York/ Philadelphia metropolitan region

L. A. BEIRN (1), B. B. Clarke (1), J. Crouch (1)
(1) Rutgers University, New Brunswick, NJ 08901
Phytopathology 98:S20

Dollar spot caused by the fungus *Sclerotinia homoeocarpa* is one of the most economically important diseases to afflict golf course turfgrass in North America. The “true” identity of the fungus has been uncertain for at least 15 years. Various taxonomic treatments have suggested the fungus may well be a member of the genus *Lanzia*, *Moellerodiscus*, or *Rutstroemia* rather than *Sclerotinia*, but none of the published literature has provided the evidence needed to pinpoint the specific identity of this organism. Similarly, knowledge of population distributions are conflicted for this fungus, and it has been suggested by some researchers that more than a single species may be responsible for the manifestation of dollar spot disease on turfgrass. To take advantage of the avalanche of molecular information that could potentially be made available to the dollar spot research community and turfgrass breeders through genomics, it is vital that we first be able to unambiguously determine the identity of this organism(s). Therefore, in this study, our primary objective is to use the genetic variability coded in the DNA of the dollar spot fungus as a tool to investigate population structuring of this fungus over a defined geographic range (New Jersey and the New York/Philadelphia metropolitan region). Here we present a summary of our findings to date, and discuss what

we've learned so far about the evolutionary processes that have shaped this important turfgrass pathogen.

Bacillus seed and boll rot of cotton: Symptoms and transmission by _Hemiptera_

A. A. BELL (1)
(1) USDA-ARS, College Station, TX
Phytopathology 98:S21

Bolls affected by seed rot and internal boll rot were sampled from various geographical areas over three years and examined for organisms capable of causing disease. After *Pantoea* species and *Nematospora coryli*, *Bacillus* species were one of the microorganisms often associated with seed and boll rot. The most common and virulent species was *Bacillus pumilus* followed by select isolates of *Bacillus subtilus*. Stink bugs collected by hand or caught in light or pheromone traps were caged over 14-day-old bolls in the greenhouse and bolls were examined and tested for the presence of pathogenic *Bacillus* species. Many stink bugs carried and inoculated cotton bolls with infectious *Bacillus* isolates. Various *Bacillus* species and isolates that have been used as biological control agents also caused seed and boll rot when puncture inoculated into bolls. These, however, were genetically distinct from most wild isolates. The ramifications of these finds will be discussed.

Classification of naturally occurring endophytes of switchgrass (*Panicum virgatum*)

J. D. BELL (1), K. D. Craven (1)
(1) The Samuel Roberts Noble Foundation, Inc., Ardmore, OK, USA
Phytopathology 98:S21

Switchgrass (*Panicum virgatum*) has emerged as a promising cellulosic bioenergy crop in the U.S., largely due to the abundance of biomass produced, its widespread N. American habitat, and the efficiency with which it utilizes both water and mineral nutrition. Symbiotic microbial endophytes are very common in grasses, as they are in most plants, and some of the best documented mutualistic plant-microbe symbioses involve cool-season grasses. Our focus is to document endophyte (both fungal and bacterial) biodiversity in native switchgrass stands in N. Oklahoma. We are utilizing both culture dependant and culture independent approaches to classify these endophytes by sequencing 16S (bacterial) and 18S (fungal) rDNA fragments and we will be presenting our methodology behind these techniques. We have compiled all data from the 2007 collection of switchgrass plants. From these results, we have decided to increase our collection to three times in 2008. We will be collecting during three periods of the plants life cycle, establishment, peak, and senescence, to be able to document the greatest microbial biodiversity in this native switchgrass stand. We will present our findings from the 2007 preliminary year's data and the data collected from the first of three rounds of collections this year. We hope to be able to utilize some of these native symbiotic endophytes to naturally enhance both abiotic and biotic stresses for the purpose of switchgrass improvement.

Sequence-directed isolation of novel bacteria contributing to soil-borne disease suppression

M. BENITEZ (1), B. B. McSpadden Gardener (1)
(1) The Ohio State University, OARDC, Wooster, OH 44691, USA
Phytopathology 98:S21

Analysis of bacterial DNA-fingerprint data followed by a sequence-directed culture-based screen resulted in the recovery of novel bacteria contributing to general soil-borne disease suppression. In previous work, *MspI*-terminal restriction fragments (TRF) of 16S rDNA were correlated with damping-off suppression. This study presents the isolation and characterization of bacteria giving rise to these TRF. Samples enriched with TRF of the target length were cloned and sequenced. Within these, sequence similarity to members of the Burkholderiales was observed in multiple M139 and M141 nt clones. Variability in the first variable loop of the 16S rRNA was used to obtain more sequence information. Primers designed for this region were used in combination with universal primers to generate ~450 nt amplicons. From these, 3 amplicon's sequence matched the initial M139 clones. Constructed sequences exhibited >97% identity to database entries in the *Genera incertae* of the Burkholderiales. Based on this sequence information, a directed culturing strategy was developed. Using this approach, a collection of ~700 bacterial isolates were obtained from soils of the suppressive cropping system. A PCR-based approach was used to screen this collection and identified 8 isolates with high levels of sequence similarity to the targeted bacteria belonging to the *Genera incertae*. Another 8 Burkholderiaceae isolates, corresponding to M141, were also identified. The isolated bacteria have the ability to reduce fungal and oomycete pathogen growth *in-vitro*, as well as to reduce disease severity in tomato and soybean seedlings. This work establishes the power of molecular screening to identify and direct the recovery of novel biocontrol strains.

Approaches to identifying functional sites in LRR domains

A. BENT (1), L. Helft (1), W. Sun (1)
(1) University of Wisconsin - Madison, Madison, WI, USA
Phytopathology 98:S21

Leucine-rich repeat (LRR) domains are prominent in the vast majority of the known plant R gene products, and in the defense-activating plant receptors for elicitors such as bacterial flagellin or EF-Tu, and in a variety of other receptor proteins. Common structural aspects of diverse LRR proteins have been identified, and diversification of LRR domains has been implicated in the evolution of new ligand specificities. However, many questions remain about how LRRs work and how they might be engineered to work differently. To address this, we have been developing experimental approaches that identify functional sites in LRR domains. Working without a solved crystal structure or a known ligand, it is possible with LRR domains to locate the clusters of predicted spatially adjacent solvent-exposed amino acids that exhibit the highest overall conservation. Alternatively, the spatially adjacent clusters that have undergone greatest diversification can also be informative. LRR consensus sequence residues are excluded from either analysis. The method appears to accurately predict key functional sites of LRRs. We have also worked to optimize the generation and testing of a limited set of site-directed mutagenesis alleles, to efficiently scan the LRR for functional sites. Diversity generation at or near these sites may generate alleles that confer altered pathogen recognition specificity. The majority of our *in vivo* testing has focused on FLS2, the flagellin receptor. Analyses of structural features of FLS2 that contribute to function will be briefly summarized.

Nested PCR is essential for the detection of extremely low titer of Candidatus Liberibacter asiaticus from citrus and its vector psyllid Diaphorina citri

L. Benyon (1), L. Zhou (2), A. Weathersbee (2), Y. DUAN (2)
(1) Dept. of Plant Pathology, University of Florida, Gainesville, FL 32653, USA; (2) USDA, ARS, USHRL, 2001 S. Rock Rd., Fort Pierce, FL 34945, USA
Phytopathology 98:S21

Citrus huanglongbing (HLB), transmitted by the psyllids Diaphorina citri and Trioza erytreae, is one of the most devastating diseases of citrus worldwide. The disease is associated with three different species of Candidatus Liberibacter: Ca. L. asiaticus (Las), Ca. L. americanus and Ca. L. africanus. Currently detection and diagnosis of HLB rely on the typical symptoms of HLB such as leaf blotchy mottle and PCR confirmation using various sets of Las-specific primers. Due to the complex nature of HLB, conventional PCR and real-time PCR were not able to detect the bacterium in certain phenotypes of the disease or from psyllids that carried a low titer of the bacterium. Three sets of primers for nested PCR were developed which targeted three different genetic loci, 16S rDNA, beta-operon and the outer membrane protein gene of the bacterium. With any one or a combination of these nested PCR Las-specific primer sets, the detection rate of Las bacterium in psyllids collected from HLB infected citrus groves was 20–30% higher than the detection rate by real-time PCR. Using nested PCR coupled with improved sampling and bacterial DNA isolation methods, we also increased detection of the Las bacterium from citrus exhibiting atypical HLB symptoms. These results demonstrated that nested PCR is essential in the detection of Las bacterium when the pathogen is present at a very low level, and it is extremely important in screening for HLB-free germplasm in citrus.

Number of insecticide sprays has no effect on the incidence of citrus huanglongbing in a commercial orchard in São Paulo, Brazil

A. BERGAMIN-FILHO (1), M. G. Gasparoto (1), L. Amorim (1), R. B. Bassanezi (2)
(1) ESALQ-USP, Piracicaba, SP, Brazil; (2) Fundecitrus, Araraquara, SP, Brazil
Phytopathology 98:S21

A total of 716,476 citrus plants (*Citrus sinensis*), from five to ten years old, distributed in 357 blocks (mean of 2006.9 trees per block) were submitted to different number of insecticide sprays (3 to 12) during three growing seasons (2004/2005, 2005/2006, and 2006/2007) in a farm located in São Paulo state, Brazil. Insecticide sprays were aimed to control citrus huanglongbing (HLB) throughout the control of its vector, *Diaphorina citri*. Eradication of symptomatic trees was carried out in the whole area 4 to 8 times per growing season. Incidences of HLB in all blocks ranged from 0.0 to 8.35% of symptomatic trees. The relationships between the number of eradicated plants and the number of insecticide sprays were investigated considering (i) each one of the three growing season, (ii) same than previous but grouping blocks according to classes of initial disease incidence, and (iii) eradicated plants in the last season (to minimize the influence of a long latent period) and the total number of insecticide sprays in all seasons. We did not find any significant negative relationship between the number of sprays and HLB incidence. We suggest that, in the conditions of the farm, the low incidence of HLB was mainly due to eradication of symptomatic trees than to insecticide sprays.

Functional analyses of three transcription factors differentially expressed during initial infection in *Magnaporthe grisea*

G. C. BERNARD (1), Y. Oh (1), J. Marui (3), T. Mitchell (2), R. A. Dean (1)
(1) North Carolina State University, Raleigh, NC, USA; (2) Ohio State University, Columbus, OH, USA; (3) Tsukuba, Japan
Phytopathology 98:S22

Magnaporthe grisea is a devastating pathogen of rice and other cereals. Studies have shown that its effect on rice (rice blast disease) causes enormous losses of production, decreasing a major food staple for many societies. To manage and potentially control this highly evolved predator, we must first understand the dynamics of its pathogenecity. Previous analyses have shown numerous genes to be differentially expressed during early stage infection. Three of these genes are; MGG_011512.5 a bromodomain containing protein first discovered to interact with the N-terminal CPKA. Bromodomains have been shown to be involved in transcriptional activation. MGG_05709.5 is a nuclear localized bHLH transcription factor with alternative transcripts expressed in appressorium formation. MGG_03196.5 contains tetratricopeptide repeats that are known to be scaffolds for protein-protein interactions. The aim of this study is to functionally characterize these genes to elucidate their roles in pathogenecity. Previous knockout analyses have shown MGG_011512.5 to be required for virulence and appressorium formation. Additional phenotyping assays will be presented as well as genetic characterization analyses for MGG_05709.5 and MGG_03196.5.

Resistance of *Pinus contorta* and *P. sylvestris* to *Gremmeniella abietina* (European race) in Sweden

A. Bernhold (1), P. Hansson (1), D. Rioux (2), M. Simard (2), G. LAFLAMME (2)
(1) Department of Forest Ecology and Management, Swedish University of Agricultural Sciences (SLU), Umeå, Sweden; (2) Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, P.O. Box 10380, Quebec (QC), Canada G1V 4C7
Phytopathology 98:S22

Gremmeniella abietina has caused severe damage to pine plantations in Europe, North America and Asia. From 2001 to 2003 in Sweden, the outbreak damaged over 480 000 ha of middle-aged *Pinus sylvestris* forests. In Canada, *P. contorta* as well as *P. banksiana* have a high level of resistance to the European (EU) race of *G. abietina*. Our objective was to test the resistance of *P. sylvestris* and *P. contorta* seedlings to the EU (large tree type) race under Swedish conditions. Trials were conducted in an opening of an infected 40-year-old *P. sylvestris* stand. After 2 years, 45% and 32% of the *P. contorta* and *P. sylvestris* seedlings were infected, respectively. However, *P. contorta* seedlings showed lower mortality, had better recovery, and the average infection length on its shoots was significantly shorter (4 cm) compared with *P. sylvestris* (10 cm). Moreover, 47% of the *P. contorta* seedlings had developed new leader shoots in 2007 compared with 19% of the *P. sylvestris* seedlings. Histopathological examinations of the transition zone of infected shoots showed that both pine species produced ligno-suberized boundaries extending from healthy needle bases transversally across the shoots. Together with phenol-filled cells, these boundaries completed the compartmentalization process in infected seedlings. These results indicate that *P. contorta* is more resistant to the EU race of *G. abietina* than *P. sylvestris*.

The presence of a functional *waaL* gene in *Erwinia amylovora* affects virulence in pear and resistance to reactive oxygen species

M. C. BERRY (1), G. C. McGhee (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI, USA
Phytopathology 98:S22

Erwinia amylovora, the causative agent of fire blight, is a gram-negative bacterial pathogen of rosaceous plants including apple and pear. Many plants employ an oxidative burst that exposes potential pathogens to harsh molecules such as hydroxyl radicals and other reactive oxygen species (ROS). *E. amylovora* is able to withstand the ROS plant response in order to colonize the host. A mutant library of *E. amylovora* strain 1189 was created using transposon mutagenesis and screened to identify mutants that were deficient in the ability to survive exposure to hydrogen peroxide. A mutant was identified with increased peroxide sensitivity and was less virulent in immature pear infection assays. The transposon insertion was in *waaL*, a gene encoding an O-antigen ligase responsible for ligating the O-antigen to the lipid A core of the lipopolysaccharide layer. Without O-antigen present, the bacterium exhibited phenotypes including a higher sensitivity to hydrogen peroxide, decreased resistance to the antibiotic polymyxin B, decreased twitching motility, and a loss of the characteristic banding pattern typically seen when looking at a complete LPS layer on a polyacrylimide gel. A *waaL* complement restored virulence in pear, decreased sensitivity to hydrogen peroxide, and restored the LPS layer.

Characterizing resistance to infection by the root pathogen *Armillaria mellea* in tolerant and susceptible grapevine rootstocks

R. BHAT (1), K. Baumgartner (2), P. Fujiyoshi (2)
(1) Department of Plant Pathology, University of California, Davis, CA, USA; (2) USDA-ARS, Davis, CA, USA
Phytopathology 98:S22

Grapevine rootstocks that are resistant to the basidiomycete fungus *Armillaria mellea* have not been identified, mainly due to lack of a rapid and reliable inoculation technique. The aim of our research was to develop an in planta assay for inoculating grapevines and tracking root infection. We propagated tolerant (Freedom) and susceptible (3309C) rootstocks from green cuttings rooted in tissue culture medium in a growth chamber at 25C. After 6 weeks, surface of the culture medium of each plant was inoculated with four mycelial agar plugs of a strain virulent on grape (n = 25 per rootstock). Within days, hyphae proliferated the medium, and by week 2, were in direct contact with roots. At 0, 2, 4, 6, and 8 weeks post-inoculation, plants were harvested (n = 5 per rootstock), roots were washed of culture medium, and pathogen growth in root tissue was confirmed by treatment with wheat germ agglutinin conjugated to a fluorophore (AlexaFluor 488) and confocal microscopy. Hyphae and roots were scanned on separate channels; hyphae appeared green, roots appeared red. Percent colonization of root area by hyphae was quantified (ImageJ, v1.37). Analysis of variance indicated significant differences in root colonization between rootstocks; 3309C had significantly higher root colonization than Freedom starting at week 4 and continuing throughout the experiment ($P < 0.0001$). The pathogen was detectable in roots, via microscopy, only two weeks after inoculation. This is a dramatic improvement over the previous inoculation technique, which resulted in earliest pathogen detection months to years after inoculation. With the ability to infect plants by *Armillaria* in a much shorter amount of time and on a reliable basis, we are now able to investigate the mode of root penetration by the pathogen, to identify susceptible portions of the root system, and to determine when foliar symptoms form in response to infection.

Detection of *Colletotrichum acutatum* in strawberry plants using nested PCR primers with enhanced specificity

R. BHAT (1), G. T. Browne (1)
(1) USDA, ARS, CPGRU, UC Davis, CA, USA
Phytopathology 98:S22

Colletotrichum acutatum incites anthracnose disease of strawberry by infecting any part of the plant. It is very difficult to ascertain the causal agent by visual and microscopic observations, because symptoms caused by *C. acutatum* resemble those of many other strawberry diseases. For good strawberry disease management, specific and quick detection of *C. acutatum* early in the growing season is beneficial. PCR-based diagnostics of *C. acutatum* and other strawberry pathogens have potential to be quick and sensitive, but they are hindered by PCR inhibitors in plant tissue and lack of pathogen-specific PCR primers. We developed a DNA extraction and purification protocol that consisted of (i) soaking the tissue pieces from diseased strawberry plants in 5% Alconox for 12 to 18 hrs, (ii) extracting DNA using a modified CTAB method, and (iii) separating the impurities electrophoretically. Using the inhibitor-free, usable total DNA from infected strawberry plant parts, we amplified the ITS1-5.8S-ITS2 region of the rDNA gene using universal primers, ITS1 and ITS4, in the first round PCR. A *C. acutatum*-specific primer pair (BColacuL127FRG and BColacuR124RRG), which specifically amplifies a 411-bp fragment from amplicons of the first round PCR, was designed and compared with the published primers in nested PCRs using target and non-target DNA. The newly designed primer pair was specific to *C. acutatum*. Upon validation of the PCR-based detection system with *C. acutatum*-specific primers using the DNA from greenhouse, nursery, and fruiting field strawberry plants infected by various pathogens including *C. acutatum*, we found that there were no false positives, and the PCR results correlated well with conventional plating assays.

Identification and evaluation of *Fusarium* species associated with root disease of soybean in Minnesota

J. C. BIENAPFL (1), J. A. Percich (1), D. K. Malvick (1)
(1) University of Minnesota
Phytopathology 98:S22

Root diseases of soybean cause substantial yield reduction in the U.S. Fusarium root rot is one of the most common, but information regarding the pathogenicity and distribution of *Fusarium* species that cause root rot of soybean is limited. In July 2007, 500 plant samples were collected from 15 fields in 10 counties representing major soybean production areas in Minnesota (MN). Symptomatic and asymptomatic root tissue was plated onto four different media. Multiple fungal genera and species were isolated; however, *Fusarium* was the most frequently isolated genus in all locations. Ten representative *Fusarium* isolates were selected from one field each in northwestern, south-central, and southeastern MN, and identified to species

using morphological characteristics and sequences of the translation elongation factor 1-alpha gene. Twenty additional isolates from plants exhibiting root rot symptoms at one location in south-central MN were identified to the species level and pathogenicity to soybean seedlings was evaluated in a greenhouse. Among the 50 *Fusarium* isolates identified were *F. oxysporum* (58% frequency), *F. solani* (14%), *F. reticulatum* (12%), *F. proliferatum* (8%), *F. equiseti* (2%), *F. graminearum* (2%), and *F. sporotrichioides* (2%). Results indicated a diversity of *Fusarium* species associated with soybean, similar to that reported in other parts of the U.S., and that at least seven *Fusarium* species are associated with soybean roots in the early reproductive stages in MN. *F. oxysporum*, *F. solani*, *F. reticulatum*, and *F. sporotrichioides* produced moderate to severe root rot symptoms on soybean seedlings and may cause root rot in MN soybean fields.

More Texas *Xylella fastidiosa* isolates colonized *Helianthus annuus* and *Iva annua* than *Ambrosia trifida* var. *texana* and *Vitis vinifera* 'Chardonnay'
M. BLACK (1), A. Sanchez (1), J. Davis (1), J. Kamas (1), P. Adams (1)
(1) Texas AgriLife Extension Service
Phytopathology 98:S23

Pierce's disease, caused by *Xyllela fastidiosa*, results in *Vitis vinifera* mortality in Texas and other southern tier states. To characterize diverse isolates, we inoculated 'Chardonnay' grape and three Asteraceae hosts with isolates from Asteraceae, woody plants, and *Vitis* species and hybrids. Cells from PWG medium (visibly turbid in SCP buffer, 0.200<OD<0.300, 600 nm) were used to twice inoculate each plant with 10-µl on two adjacent internodes (20 µl/date, 40 µl total) and sterile syringe probes. Repeat inoculations (1 to 3 days later) were on opposite sides of internodes. Controls were SCP buffer. Inoculation site and a distal non-inoculated internode were ELISA tested after 10–18 weeks. Most grape isolates induced grape symptoms and were ELISA positive but non-grape isolates rarely induced minor symptoms or were ELISA positive. Weeds did not develop symptoms. Most isolates in *Helianthus annuus* (annual sunflower), *Iva annua* (seacoast sumpweed) and *Ambrosia trifida* var. *texana* (giant ragweed) tested ELISA positive. *H. annuus* was most compatible with isolates from Asteraceae, then similarly compatible with isolates from *Vitis* and woody species. *I. annua* was most compatible with isolates from Asteraceae, then *Vitis*, then woody species. *A. trifida* var. *texana* was most compatible with isolates from Asteraceae, then woody and then *Vitis* species. *V. vinifera* was clearly most compatible with *Vitis* isolates, and had low or no compatibility for isolates from Asteraceae and woody species. Three Asteraceae species near vineyards may harbor *X. fastidiosa* pathogenic to grape as minority members of mixed infections. Site selection and vegetation management are indicated to avoid and minimize these Asteraceae species.

***Phytophthora* genomics: Identifying new markers for population-level studies**
J. E. BLAIR (1)
(1) Amherst College, Amherst, MA
Phytopathology 98:S23

Traditionally, genetic diversity within *Phytophthora* species has been examined using one or a few molecular tools, such as single gene sequencing, isozymes, or RFLPs. A robust phylogeny for the genus has recently been established using molecular markers derived from complete genome sequence data. Additional markers are now being identified from genomic sequences to study genetic diversity within populations and species complexes. Here I will present preliminary data on five studies which have used information from complete genomes to develop new population- and species-specific molecular tools. Two studies investigate genetic diversity within species complexes using intron-containing coding sequences, and three studies use random genomic survey sequence (GSS) libraries. Technical challenges for generating and analyzing data from a large number of isolates will also be discussed.

Genetic structure of populations of the tobacco blue mold pathogen, *Peronospora tabacina* in North America, Central America and the Caribbean and Europe
M. BLANCO-MENESES (1), I. Carbone (1), K. Ivors (1), J. B. Ristaino (1)
(1) North Carolina State University
Phytopathology 98:S23

Tobacco blue mold, caused by the oomycete pathogen *Peronospora tabacina* causes a yearly epidemic in tobacco (*Nicotiana tabacum*). The genetic structure was examined by sequencing specific genes and spacers including: mitochondrial *cox2*, P3 (*rpl*14, *rpl*5, tRNA), and nuclear IGS2 and *ypt1*. For the IGS2 region 326 clones and 140,832 nucleotides were sequenced from populations from burley and flue-cured isolates, North Carolina (NC), the U.S., Central America-Caribbean-Mexico (CCAM) and Europe (EU). Two different transition events and 1 transversion event separated these populations into 8 frequent haplotypes. There were also 116 less frequent haplotypes. Nucleotide diversity was 2.986 in NC, 2.808 in the U.S., 3.484 in CCAM, and 2.823 in EU. The average per-nucleotide expected heterozygosity (Watterson's theta) was 22.161 for all regions, 9.398 for NC, 15.144 for the U.S., 11.76 for CCAM, and 6.724 for EU. Neutrality tests were significant for all populations and the equilibrium model of neutral evolution was rejected, indicating an excess of recent mutations or rare alleles. Hudson's tests were performed to quantify genetic structure within and between populations. Burley and flue-cured populations were not subdivided. Population subdivision was also not observed between all 4 regions. Migration was observed between Mexico, the Dominican Republic and Guatemala but not Nicaragua. Population subdivision was also not observed between the U.S. and CCAM or within U.S. populations. Migration between the Dom. Republic and Georgia, NC and Virginia was observed. Migration was also observed between European and Lebanon tobacco fields.

Regulation of pathogenesis by light in *Cercospora zeae-maydis*: Identification of a photoreceptor required for infection of maize
B. H. BLUHM (1), L. D. Dunkle (2)
(1) Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA; (2) USDA-ARS Crop Production and Pest Control Research Unit, Purdue University, West Lafayette, IN 47907, USA
Phytopathology 98:S23

Cercospora zeae-maydis causes gray leaf spot, one of the most prevalent and damaging foliar diseases of maize throughout the world. Light plays a key role in the virulence of *C. zeae-maydis* by inducing the biosynthesis of cercosporin, a photosensitizing, non-host-specific phytotoxin. We determined that blue light induces cercosporin biosynthesis but represses conidiation in *C. zeae-maydis*. To understand how light regulates pathogenesis at the molecular level, we identified and characterized two genes encoding putative blue-light receptors in the fungus: Phl1, a putative member of the cryptochrome-photolyase family of photoreceptors, and Crp1, an ortholog of White Collar-1 from *Neurospora crassa*. Disruption of *PHL1* abolished photoreactivation, the light-induced DNA repair after exposure to lethal doses of UV light, but had no discernible effect on the ability of the fungus to infect and colonize maize leaves. Disruption of *CRP1*, however, nearly eliminated the ability of the fungus to form appressoria over stomata, thus rendering the mutants virtually non-pathogenic. Surprisingly, disruption of *CRP1* neither abolished the production of cercosporin nor fully derepressed conidiation in blue light, suggesting the existence of a novel, undescribed fungal blue-light photoreceptor. Based on our findings, we propose a working model to explain the molecular regulation of pathogenesis in *C. zeae-maydis* in response to light.

Characteristics of multi-rater estimates of citrus canker severity
C. H. BOCK (5), P. E. Parker (2), A. Z. Cook (3), T. Riley (4), T. R. Gottwald (1)
(1) USDA, 2001 S. Rock Rd., Ft. Pierce, FL 34945; (2) USDA-APHIS-PDDML, 22675 N. Moorefield Rd., Edinburg, TX 78539; (3) USDA-APHIS-PPQ, 22675 N. Moorefield Rd., Edinburg, TX 78539; (4) USDA-APHIS-PPQ, 800 Thorpe Road, Orlando, FL 32824; (5) University of Florida, 2001 S. Rock Rd., Ft. Pierce, FL 34945
Phytopathology 98:S23

Citrus canker (CC, caused by *Xanthomonas citri* subsp. *citri*) was under eradication for 10 y in Florida. A total of 28 CC surveyors and plant pathologists rated severity of CC symptoms on 200 images to investigate the range of abilities and some factors that influence canker severity estimation. Actual disease was measured on each leaf image by image analysis. Estimates of lesion number (LN) were most accurate and precise, (r^2 = 0.88–0.98), followed by % area necrotic+chlorotic (NC, r^2 = 0.63–0.93) and % area necrotic (N, r^2 = 0.44–0.94). Raters over- or under-estimated disease, and displayed heterogeneity of variance. Lin's concordance analysis showed characteristics of individual error in precision and accuracy due to location and scale shifts, and tests for heteroscedasticity (White's and Breusch-Pagan) confirmed it was ubiquitous with actual disease severity ($P < 0.0001$). Averaging raters estimates provided an accurate and precise mean estimate (r^2 = 0.97, 0.87 and 0.92 for LN, N and NC, respectively). LN influenced most raters causing over-estimation of disease area, especially apparent at low severity. A small number of raters also showed a tendency to overestimate due to LN across the spectrum of severity. Although averaging severity scores gave an accurate and precise mean estimate, the rater, and various aspects of the symptoms affect accuracy and precision of individual estimates. Training assessors should improve estimation of CC severity estimation.

Disadvantages of the Horsfall-Barratt scale for estimating severity of citrus canker
C. H. BOCK (3), T. R. Gottwald (1), A. Z. Cook (2), P. E. Parker (2)
(1) USDA, 2001 S. Rock Rd., Ft. Pierce, FL 34945; (2) USDA-APHIS-PPQ, 22675 N. Moorefield Rd., Edinburg, TX 78539; (3) University of Florida, 2001 S. Rock Rd., Ft. Pierce, FL 34945
Phytopathology 98:S23

Estimates of citrus canker (caused by *Xanthomonas citri* subsp. *citri*) severity are important to studies of disease epidemiology, management and breeding

for resistance. Two methods, the Horsfall-Barratt (H-B) scale and direct estimation, were compared for estimating disease severity on 200 images of known infection (0–37% leaf area infected). Each leaf image was assessed by 28 raters. A linear relationship was found between actual and estimated disease for all raters. Estimates of disease varied in accuracy and precision by both direct and H-B estimation. More replication generally resulted in greater accuracy and precision of the mean estimate – for both direct and H-B estimation. Direct estimation provided a more consistent estimate of severity at low replication (r^2 = 0.78 and 0.68 for means of 3 replicates by direct and H-B estimation, respectively), 5 replicate estimates were more consistent (r^2 = 0.87 and 0.84, respectively) and 28 replicate estimates resulted in most consistency (r^2 = 0.93 and 0.92, respectively). Variance of 3 replicate estimates by direct rating was more consistent (r^2 = 0.63) compared to H-B (r^2 = 0.43). Consistency was improved for both methods with 5 replicates (r^2 = 0.74 and 0.67, respectively) and with 28 replicates (r^2 = 0.92 and 0.91, respectively). The H-B inter-conversion did not improve accuracy and precision or variance compared to direct estimation. At low replications H-B was detrimental to estimates of the mean and variance.

Comparative transcript profiling of *Lr1*- and *Lr34*-mediated leaf rust resistance in wheat

M. D. BOLTON (3), J. A. Kolmer (2), W. W. Xu (1), D. F. Garvin (3)
(1) Supercomputing Institute, University of Minnesota, Minneapolis, MN, USA; (2) USDA - ARS, Cereal Disease Laboratory, St. Paul, MN, USA; (3) USDA - ARS, Plant Science Research Unit, St. Paul, MN, USA
Phytopathology 98:S24

Leaf rust caused by the fungus *Puccinia triticina* is a widespread disease of wheat. Host resistance strategies to control leaf rust have relied upon race-specific and non-race specific leaf rust resistance (*Lr*) genes. Although race-specific *Lr* genes are efficient in halting pathogen growth, high levels of genetic variation in *P. triticina* often lead to rapid decline in their effectiveness. In contrast, non-race specific *Lr* genes confer durable resistance to *P. triticina* and condition a partial resistance phenotype that slows pathogen proliferation. This study compared changes in wheat gene expression associated with 1) a compatible interaction between wheat and *P. triticina*, 2) resistance encoded by a typical race-specific resistance gene *Lr1*, and 3) race non-specific resistance encoded by *Lr34*. Thatcher wheat and near-isolines harboring either *Lr1* or *Lr34* were inoculated with *P. triticina* and transcript profiles were assessed at two timepoints using the Affymetrix wheat GeneChip. Surprisingly, no differentially expressed genes were detected in *Lr1* plants at either timepoint, while in the compatible interaction differentially expressed genes were detected only at 7 dpi. Genes upregulated in the *Lr34* line included those encoding defense and stress-related proteins, secondary metabolism enzymes, and genes involved in transcriptional regulation and cellular signaling. Our results also indicate that the *Lr34*-mediated defense response is energetically costly, based on coordinated up-regulation of genes in several aerobic and anaerobic primary metabolic pathways that provide a means to produce more ATP. Thus, at the molecular level the modes of action of the leaf rust resistance genes *Lr34* and *Lr1* are distinctly different.

Impact of plant activators and copper on bacterial speck and host response in field-grown tomatoes

M. A. BORSICK HERMAN (1), H. W. Lange (1), S. L. Davidson (2), C. D. Smart (1)
(1) Cornell University, Geneva, NY, USA; (2) Hobart and William Smith Colleges, Geneva, NY, USA
Phytopathology 98:S24

Activating plant defenses is a promising disease management option. Two types of plant activators and copper were evaluated for control of bacterial speck disease, effect on foliar pathogen populations and tomato defense gene activation over two field seasons. Acibenzolar-*S*-methyl (ASM), which activates systemic acquired resistance, controlled *Pseudomonas syringae* pv. *tomato* as well as copper and without negatively affecting yield. The plant growth-promoting rhizobacteria (PGPR, activator of induced systemic resistance) compound reduced bacterial speck symptoms relative to the untreated control though did not consistently control to the level of copper and ASM. Pathogen growth and plant defense responses were followed using quantitative real-time PCR on leaf samples collected from the field. All treatments negatively impacted pathogen growth. Response of ASM-treated plants was dependent on disease pressure; the salicylic acid and ethylene-mediated responses were detectable only under high disease pressure. Despite providing some disease control, no priming of marker gene expression was observed in PGPR-treated plants. Implications of these findings on field management strategies are discussed.

Response of native potatoes from the Venezuelan Andes to the infection by *Phytophthora infestans*

K. BOSCAN (1), A. Briceño (1), G. A. Fermin (1)
(1) Universidad de Los Andes, Merida, Merida, Venezuela
Phytopathology 98:S24

In Venezuela, the potatoes are an important component of its population diet. The potato crops date back to the prehispanic era in the Andes of Bolivia, Chile and Peru, as well as in Venezuela, where their true origin is uncertain. Alike many other crops, the potato, when exploited massively, is subject to the attack of diverse pathogens, among which the most devastating and economically important is the oomycete *P. infestans*, the causal agent of potato late blight. Chemical control is so far the only way to deal with the disease, which besides being tremendously harsh to the environment increases production costs, and constitutes a potential source of poisoning in countries where agrochemicals are applied indiscriminately. The potatoes that are currently cultivated in the country are derived from imported seed, employing cultural practices that include chemical control. The Venezuelan native potatoes receive different names according to the region they have been relictually maintained. Based on production cycles and morphological data, there seemed to be a high degree of variability in our native potatoes, regarded by the producers as resistant to late blight. In an attempt to characterize this germplasm, and find potential sources of resistance against *P. infestans*, different molecular markers were used to assess the identity and degree of variability among Venezuelan native potatoes. After characterizing this germplasm, 12 different isolates of *P. infestans*, from our collection of more than 600 single lesion isolates, were used in infection-controlled experiments with detached potato leaflets. Compared to the commercial potatoes, some of the Venezuelan native potatoes no only showed a good level of resistance to the pathogen, but also a high degree of variability which points to a wide genetic basis useful for the development of a potato improvement program.

Impact of winter cover crop and planting data on diseases, yield and aflatoxin contamination of peanut

K. L. BOWEN (1), A. K. Hagan (1), H. Campbell (1)
(1) Dept. Entomology and Plant Pathology, Auburn University, Auburn, AL, USA
Phytopathology 98:S24

Dryland peanut, which was planted following rye, oats, wheat or winter fallow (bare soil), was sown at four planting dates. Each of four replicate plots was sampled for a number of diseases and pod samples from each plot were assayed for aflatoxin contamination. Despite hot, dry summer weather through the 2007 growing season, levels of aflatoxin contamination were low (0 to 89 ppb; average = 15.6 ppb) in sampled peanuts. Samples from peanuts planted following winter wheat tended to have the lowest levels of contamination, while those from oats tended to have higher levels of contamination. Root-knot nematode damage ratings from individual plots were correlated ($P < 0.005$) to incidence of stem rot (caused by *Sclerotium rolfsii*) as well as aflatoxin contamination of peanuts.

Effect of pyraclostrobin foliar fungicide on multiple corn hybrids

C. A. BRADLEY (1), D. G. White (1), K. W. Campbell (2)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL, USA; (2) Monsanto Company, Johnston, IA, USA
Phytopathology 98:S24

In 2007, foliar fungicides were applied to approximately 4 to 6 million hectares of corn (*Zea mays*) in the Midwestern United States (U.S.). This was the most corn ever sprayed with a foliar fungicide in the U.S. Historically, use of foliar fungicides in corn have been limited to seed production fields, where profit margins are greater; however, the increasing market price of corn has allowed the use of more inputs in corn production such as foliar fungicides. Because corn hybrids vary in their level of resistance to foliar diseases such as gray leaf spot, caused by *Cercospora zeae-maydis*, a study was initiated to evaluate the response of multiple hybrids to pyraclostrobin foliar fungicide (Headline, BASF Corp., Research Triangle Park, NC) at three locations (Champaign, IL; Lebanon, IN; and Williamsburg, IA). In 2007, 34 to 40 hybrids per location were either left untreated or sprayed with pyraclostrobin at 110 or 165 g a.i./ha. Fungicides were applied when hybrids were in the VT to R1 developmental stages. At all locations, the main effects of hybrid and fungicide were significant ($P \leq 0.05$) on yield; however, the hybrid × fungicide interactive effect on yield was significant only at the Williamsburg location. At Champaign, only pyraclostrobin at 110 g a.i./ha significantly increased yield over the untreated control (increase of 251 kg/ha). At Lebanon, pyraclostrobin at 110 and 165 g a.i./ha significantly improved yield over the untreated control by 1,570 and 1,821 kg/ha, respectively. At Williamsburg, hybrids with less resistance to gray leaf spot tended to respond greater to fungicides, with yield increases over the untreated control ranging from 63 to 2,261 kg/ha.

The effect of nitrogen, sulfur and fungicide applications on the severity of necrotic ring spot of Kentucky bluegrass

N. Brandt (1), A. Koski (1), N. TISSERAT (1)
(1) Colorado State University
Phytopathology 98:S25

Necrotic ring spot (NRS), caused by *Ophiosphaerella korrae*, is the most serious disease of Kentucky bluegrass lawns in Colorado. We studied the influence of fertilizer rates, formulation and timing, and fungicide applications on NRS severity. Urea and urea plus sulfur (U, U+S) were applied in spring and fall to a diseased residential lawn beginning in October 2005. A urea-based, timed-release (TR) fertilizer was applied once to the same lawn in April of 2006 and 2007. The three N formulations were applied at a rate of 98 and 196 kg ha^{-1} yr^{-1}. Azoxystrobin (Heritage) was applied in mid-May and mid-July each year. There were no differences in NRS severity between fertilizer rates on any rating date. However, NRS was more severe in TR fertilizer plots in both years. In subsequent studies, we observed the highest nitrogen content in leaves in May following the TR application. Application of sulfur with urea suppressed NRS in the second year compared to urea applications alone. Two applications of azoxsytrobin reduced NRS severity in August of both years.

Evolving management strategies for *Monilinia fructicola* populations with reduced sensitivity to DMIs in Georgia and implications for brown rot control recommendations

P. M. BRANNEN (3), K. Taylor (4), M. Hotchkiss (2), C. Reilly (2), G. Schnabel (1), A. Amiri (1)
(1) Clemson University, Clemson, SC, USA; (2) USDA-ARS, Byron, GA, USA; (3) University of Georgia, Athens, GA, USA; (4) University of Georgia, Byron, GA, USA
Phytopathology 98:S25

Brown rot of peach, caused by *Monilinia fructicola*, is a major disease in Georgia, and sterol demethylation inhibitor (DMI) fungicides have been utilized extensively for control of this pathogen. A survey conducted in 2004 indicated that *M. fructicola* populations with reduced sensitivity to propiconazole were generally present throughout the middle Georgia peach production region. Results from several field trials have indicated that stand-alone use of DMI fungicides at currently recommended rates will result in severe losses. Two preharvest applications of either boscalid + pyraclostrobin or boscalid + pyraclostrobin followed by propiconazole have provided good control in the presence of DMI-insensitive *M. fructicola* populations, as have two preharvest applications with increased rates of fenbuconazole. The latter indicates that DMI-insensitive *M. fructicola* populations can be managed by increasing rates of DMI fungicides. Preharvest application of calcium chloride and tank-mixtures of propiconazole with captan or sulfur have also been reviewed, and thus far these have met with limited success in augmenting disease management.

Intraspecific group of *Rhizoctonia solani* AG 2-2 and rotation crop affect sugar beet

J. BRANTNER (1), C. Windels (1)
(1) University of Minnesota, NW Res & Outreach Ctr., Crookston, MN, USA
Phytopathology 98:S25

Rhizoctonia solani AG 2-2 IV and IIIB are pathogenic to sugar beet in Minnesota and North Dakota. In 2005 and 2006, plots were infested with IV, IIIB, and not-infested (control) and sown to wheat, soybean and corn. At harvest, isolation of *R. solani* from roots of wheat, soybean and corn in the control averaged 1, 4 and 2%, respectively; IV plots averaged 2, 12 and 5%, respectively; and IIIB plots averaged 5, 16 and 33%, respectively. When sugar beet was sown in 2006 and 2007, Rhizoctonia damping-off occurred 3 wk later; stands were highest in the control regardless of previous crop, lowest in IIIB plots sown with soybean and corn, and intermediate and equal in soil with IV after all crops and in soil with IIIB after wheat. At harvest, Rhizoctonia root and crown rot (RRCR) was more severe in 2006 than 2007 but overall, was highest in plots with IIIB, lowest in the control, and intermediate in plots with IV; RRCR ratings were highest after corn, lowest after wheat, and intermediate after soybean. In 2006, root and sucrose yields (averaged across all plots) were significantly higher following wheat and equally low following soybean and corn; in 2007, these variables were not significantly different but tended to be higher after wheat and soybean and lower after corn. Thus, ISG of *R. solani* AG 2-2 and previous crop can affect severity of RRCR on sugar beet.

Correlation of ear rot incidence with insect damage in Bt corn hybrids

K. N. BRAUER (1), R. J. Wright (1), T. A. Jackson (1)
(1) University of Nebraska-Lincoln
Phytopathology 98:S25

Ear rotting fungi are a common occurrence in field corn. While they may not drastically reduce yield, they can contaminate grain with mycotoxins, for which producers can be severely penalized. Ear feeding insects play an important role in fungal colonization of the corn ear. Bt corn hybrids theoretically reduce insect damage which should help mitigate field infections and reduce mycotoxin contamination. Field trials were established at two locations in 2007 to test this hypothesis. Two hybrid lines were chosen from Dekalb and Pioneer including DKC60-17, DKC60-18, DKC60-19, 34A14, 34A17, and 34A20. These were planted on May 10 and 23 at Mead and May 11 and 29 at Clay Center. Staggered planting dates were used to generate a varying degree of insect pressure and damage and fungal infection. At crop maturity, ears were manually harvested and the incidence of insect injury and visible fungal infection was determined. Fungal infection was positively correlated with insect damage in the later planting date at both locations ($P \leq 0.0001$). Brand, hybrid, and insect resistance traits did not significantly affect yield at either location except for insect resistance traits in the latest planting at Clay Center.

Investigating the threat of *Phytophthora ramorum* to Ireland: The current situation

J. BRENNAN (3), D. Cummins (3), S. Kearney (3), J. Choiseul (3), G. Cahalane (1), S. Nolan (2)
(1) Forest Service, Department of Agriculture, Fisheries & Food, Kildare Street, Dublin 2, Ireland; (2) Horticulture & Plant Health Division, DAFF, Maynooth, Co. Kildare, Ireland; (3) Plant Health Laboratory, Backweston Campus, Department of Agriculture, Fisheries & Food, Celbridge, Co. Kildare, Ireland
Phytopathology 98:S25

Phytophthora ramorum is a serious pathogen of trees and ornamental plants, causing a disease known as sudden oak death (SOD) in the USA, where it has had major environmental & economic impacts. Plants affected by *P. ramorum* show a range of symptoms such as leaf blight, stem canker, and tip dieback. The pathogen was first detected in the USA in the mid 1990s, Europe 2001 (it was first named in 2001 but the disease was first detected in early 1990s) and Ireland in 2003. *Phytophthora ramorum* is subject to plant health controls in the EU under Commission Decision 2002/757/EC (& amendments). The organism has been isolated from over 30 plant species worldwide including a number which have significant commercial and amenity value in Ireland, particularly *Rhododendron* and *Viburnum* spp. To date *P. ramorum* has not been detected on tree species in Ireland, however there is strong concern however that Irish trees could become infected. Extensive surveys have been carried out by the Department of Agriculture, Fisheries & Food (DAFF) from 2003 to present. Since 2003 over 4000 samples were collected from nurseries, garden centres, public parks and forests around Ireland. *Phytophthora ramorum* was detected in all years [positive samples: 8% (2003), 2% (2004), 19% (2005), 10% (2006) & 16% (2007)]. In 2003, *P. ramorum* was only found on *Rhododendron* and *Viburnum* spp., however by 2007 the presence of *P. ramorum* was confirmed on five plant genera (*Rhododendron*, *Viburnum*, *Camellia*, *Photinia* & *Magnolia*). *Phytophthora ramorum* has also been found on wild *Rhododendron* spp. On going surveys are being carried out by Horticulture and Plant Health Division and the Forest Service of DAFF. Eradication & containment measures are being implemented in accordance with EU legislation.

Phylogeography and sequence diversity of genetic lineages of the grapevine powdery mildew fungus, *Erysiphe* (*Uncinula*) *necator*, in North America, Europe, and Australia

M. T. BREWER (1), M. G. Milgroom (1)
(1) Dept. of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853
Phytopathology 98:S25

Plant pathogens have evolved in response to pressures in natural systems and changes in host characteristics that accompanied crop domestication; and they continue to evolve in response to current agricultural practices. An understanding of the processes leading to the evolution of new pathogens through the formation of new species—a process known as speciation—is important in predicting in the emergence of novel pathogens. *Erysiphe necator* provides a unique opportunity to study incipient speciation since two distinct genetic lineages have been detected on *Vitis vinifera* throughout Europe and Australia. Yet, the genetic structure of *E. necator* in eastern North America, the proposed origin and assumed center of diversity, is unknown. We used a multilocus gene genealogy approach to understand the diversity and phylogenetic structure of populations from North America in relation to the genetic groups found in Europe and Australia. Five gene regions were sequenced from approximately 100 isolates obtained from both wild and cultivated *Vitis* spp. and *Parthenocissus* spp. throughout eastern North America, and from cultivated *V. vinifera* from California, Oregon, France, Italy, and Australia. Results indicate that although genetic groups A and B were not found in eastern North America, they diverged from eastern North American ancestors. Populations from wild or cultivated grapes in the eastern

U.S. are not genetically distinct; however, isolates from *Parthenocissus* are strongly divergent suggesting that they represent a separate species. Nucleotide sequences are most diverse for the eastern North American samples. Genetic group B was detected in California and Oregon and may have been introduced on cultivated *V. vinifera* from Europe. Knowledge of the genetic structure, as well as biological and epidemiological differences between groups of this fungus, is essential for sound management of grapevine powdery mildew.

Effect of soil physical properties on incidence of corn and soybean damping-off caused by *Pythium* spp.
K. D. BRODERS (1), M. Wallhead (1), G. Austin (1), P. A. Paul (1), P. E. Lipps (1), A. E. Dorrance (1)
(1) The Ohio State University, OARDC, Wooster, OH
Phytopathology 98:S26

Multiple *Pythium* spp. are responsible for causing pre- and post-emergence damping-off of both corn and soybeans. The role of soil physical properties, relating to percent sand, silt, and clay, was evaluated using samples collected from 88 different production fields in Ohio. The locations selected were fields where either low, moderate, or high levels of seedling disease had occurred previously; and represented the five major soil regions where corn and soybeans are grown in the state. The soil collected was used in a baiting procedure, and disease incidence was scored as the percent of infected plants and percent healthy stand. The percent infected plants from each location ranged from 20 to 85%, with a mean of 49%. Cluster analysis of percent sand, silt, and clay arranged the locations into four clades. The clusters were significantly different ($P < 0.05$) for disease severity based on ANOVA. Soils from locations with a greater proportion of silt and clay had higher levels of disease incidence than soils with a higher proportion of sand. These findings indicate the importance of soil physical properties contributing to the incidence of disease caused by *Pythium* spp., and suggest that management recommendations for corn and soybean seedling disease may be more accurate if based on soil physical properties and not soil regions.

Assessing *Pythium* population dynamics from different soil regions in Ohio
K. D. BRODERS (1), M. Wallhead (1), P. A. Paul (1), P. E. Lipps (1), A. E. Dorrance (1)
(1) The Ohio State University, OARDC, Wooster, OH
Phytopathology 98:S26

The number of *Pythium* spp. which can infect corn and soybean may be far greater than previously thought. Our lab has adapted a high-throughput process to expedite the identification of over 6000 isolates of *Pythium* from 88 locations representing five soil regions in Ohio. The objective of this study was to evaluate the diversity of *Pythium* spp. causing corn and soybean seedling disease and relate this diversity to soil physical and chemical properties. There were 18 species and 2 putative new species identified, and in most locations, one or two species represented greater than 60% of the isolates recovered. Species diversity was measured using the Shannon index and varied from 0.2 to 2.0. A cluster analysis grouped locations based on soil chemical properties, and an ANOVA was performed to evaluate the difference in species diversity between the clusters. Locations with relatively low species diversity had a low cation exchange capacity, low organic matter, and clay was >30% and also had higher disease incidence. Locations with higher species diversity had higher levels of phosphorus, potassium, and calcium, and organic matter and had lower levels of disease incidence. These results suggest that diversity of pathogenic *Pythium* spp. is limited by nutrient poor soils with limited drainage and disease incidence is favored by these same factors.

Resistance Screening Center, USDA Forest service forest health protection unit, Asheville, NC
J. J. BRONSON (1)
(1) USDA Forest Service, Asheville, NC, USA
Phytopathology 98:S26

The Resistance Screening Center evaluates seedling materials for resistance to disease, primarily fusiform rust (*Cronartium quercuum* f. sp. *fusiforme*) and pitch canker (*Fusarium circinatum*) as a service to tree improvement specialists, seed orchard managers, research institutions, and others in private industry and government agencies. Testing enables clients to obtain information on the relative resistance of materials, in much less time than is possible in field progeny tests. By using information from these tests, trees producing disease resistant progeny are identified; or questions may be answered concerning such things as the nature of the pathogen or effectiveness of fungicides. The RSC can modify current screening procedures so as to allow researchers to utilize the facilities as an additional experimental tool. In a research assistance capacity, the RSC has played an important role in newly developed understanding of genetic interactions in the fusiform rust pathosystem. The Forest Service has an alternate interest in screening the material submitted for disease resistance to the Resistance Screening Center. The use of resistant materials on private land can reduce the base from which these pathogens might spread to Federal, State, or other private land.

Genotype × management interactions influence susceptibility to false smut and kernel smut of rice
S. BROOKS (1), M. Anders (2)
(1) USDA ARS, Stuttgart, AR, USA; (2) University of Arkansas Rice Research and Extension Center, Stuttgart, AR, USA
Phytopathology 98:S26

False smut (*Ustilaginoidea virens*) and kernel smut (*Neovossia horrida*) have historically been considered minor diseases of rice (*Oryza sativa*) in the United States. The two diseases are often consolidated with regard to importance, resistance and control, leading to ambiguous assessments of these unique pathogens. False smut is rapidly growing in importance internationally and has replaced blast as the most importance disease of rice in parts of China. Kernel smut is a very different disease that is often undetected due to partial bunting of the kernel that is concealed by the hulls (palea and lemma). Kernel smut impacts the parboiling industry where rice is cooked in the hulls, and spore release discolors kernels of the finished product. Our goals were to clearly identify agronomic practices that influence each disease (GxE), identify genetic resistance that is effective across environments, and optimize agronomic practices to maximize returns from high-yielding albeit disease susceptible rice varieties. Using disease nurseries established in Stuttgart Arkansas the effects of fertilizer rate, crop rotation, tillage and irrigation practices on disease incidence and yield in rice varieties were evaluated. Disease incidence was reported as number of sori (false smut) or smutted kernels (kernel smut) per kilogram of paddy rice from combine harvested research plots. All treatments had significant effects on one or both diseases, but each disease responded independently to the treatments. Differences in susceptibility were observed among cultivars, and fertility treatments were also significant for both diseases. Tillage, rotation and irrigation were all significant treatments influencing cultivar susceptibility to false smut. Significance: sources of genetic resistance to kernel smut were identified, as well as agronomic practices that reduce the incidence of false smut on susceptible cultivars.

Evaluation of the Rossi et al. 2000 apple scab ascospore release model in California pear orchards
J. C. BROOME (1), W. Gubler (3), C. Ingels (1), R. Elkins (1), J. F. Strand (2)
(1) University of California Cooperative Extension; (2) University of California Integrated Pest Management Program; (3) University of California, Davis
Phytopathology 98:S26

Pear scab, caused by the fungus *Venturia pirina*, is an important disease in California. Several weather-driven models have been developed for apple and pear scab that describe different stages in disease development. We tested an Italian apple scab model, Rossi et al. 2000, to see if it could be used to predict ascospore capture in California pear orchards. Temperature and hours of leaf wetness are used to calculate a wetness-based degree day model (above 0 C, or 32 F) that predicts the Proportion of Ascospores Trapped (PAT). We developed a user-friendly MS excel spreadsheet version of the Rossi et al. 2000 model. We conducted preliminary tests of the model using archival weather and Burkart 7-day continuous-run vacuum spore trap data from the 1998 and 1999 seasons in Potter Valley and Talmage, Mendocino County, California. Preliminary analysis shows that the Rossi et al. 2000 model predicted ascospore captures very well in the 1999 season at both locations, but was less accurate in predicting ascospore captures in the 1998 season. In 1998, the model was late in predicting the increase in the proportion of spores trapped by several days to up to 3 weeks. This happened at both the beginning and the ending of the ascospore release season. The 1998 growing season was a particularly bad pear scab year, with prolonged, cold wet weather early in the season, which made orchard access limited and disease pressure very high. Further statistical analyses to test for the model's goodness of fit are currently being conducted and will be reported. Ascospore trappings (dependent variable) will be regressed against model estimates (independent variable) and the properties of the linear model will be examined.

Investigation of maize kernel proteins for use as markers for newly developed aflatoxin-resistant inbreds
R. L. BROWN (3), A. Menkir (1), Z. Chen (2), R. Bandyopadhyay (1), M. Luo (2), T. E. Cleveland (3)
(1) International Institute of Tropical Agriculture, Ibadan, Nigeria; (2) Louisiana State University Agricultural Center, Baton Rouge, LA, USA; (3) USDA-ARS-SRRC, New Orleans, LA, USA
Phytopathology 98:S26

Six maize inbreds with resistance to aflatoxin contamination developed through an international breeding program in Nigeria are being registered for release to the public. These lines are the result of original crosses between

U.S. and African lines and were selected not only for demonstrated resistance, but also for good agronomic characteristics. Proteomic investigations comparing resistant with susceptible maize lines have been conducted using the domestic parents of the breeding program as subjects and also breeding materials generated from the program. Proteins associated with resistance, ones upregulated in resistant lines, have been identified and fall into four categories: 1) antifungal; 2) storage; 3) stress-related; and 4) putative regulatory. Of these, several have been investigated further using physiological and biochemical methods, including RNAi gene silencing. Results of investigations are discussed; projections for future studies are advanced.

A begomovirus and suite of satellites associated with the leaf curl diseases of tomato and tobacco from Yemen are evolutionarily most closely related to begomoviruses from the Nile Basin

J. K. Brown (2), N. M. Abdullah (1), A. M. IDRIS (2)
(1) Department of Plant Protection, Sana'a University, Sana'a, Yemen; (2) Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA
Phytopathology 98:S27

Leaf samples were collected from tobacco and tomato plants exhibiting yellowing, leaf curling, and stunting symptoms from Tehama, on the western coast of Yemen. Several full-length begomoviral and a suite of satellite DNA (satDNA) molecules were amplified by rolling circle amplification (RCA) from total nucleic acids isolated from tobacco and tomato leaves, cloned, and the DNA sequence was determined. The assembled nucleotide (nt) sequences for the tomato (n = 1) and tobacco (n = 1) isolates revealed the presence of a single begomoviral genome of 2,781 nt. Attempts to detect a DNA-B component by RCA or polymerase chain reaction were unsuccessful. The ~2.8 kb genome, genome organization, and absence of a DNA-B component indicated that the virus is monopartite. Nt sequence comparisons indicated that it shared 89.6% identity with the *Tomato leaf curl Sudan virus* Shambat (ToLCSDV-Sha), and herein is designated ToLCSDV-Ye. ToLCSDV-Ye shared its' second highest nt identity, at 86.8%, with the Gezira strain of ToLCSDV (ToLCSDV-Gez), also from Sudan. Fourteen full-length satDNAs (1.3 kb) were cloned and sequenced from tomato (n = 6) and tobacco (n = 8) extracts. The satDNA nt sequences were 99–100% identical, indicating a single, predominant satDNA type. Unexpectedly, the ToLCSDV-Ye satDNA shared <40% nt identity with the Asian and Nile Basin beta satDNA clades, respectively. In contrast to ToLCSDV-Ye, which grouped with the Nile Basin begomovirus clade, the ToLCSDV-associated satDNA represents a new, divergent beta satDNA clade prototype. The ToLCSDV-Ye beta satDNA exhibited all the hallmarks of other begomovirus-associated beta satDNAs: one open reading frame, several predicted hairpins, and an adenine-rich and a conserved region, respectively.

A model system for measuring citrus propagation risk mitigation based on Hazard Analysis and Critical Control Point (HACCP) methods

L. G. BROWN (1)
(1) USDA APHIS PPQ Center for Plant Health Science and Technology, Raleigh, NC 27606
Phytopathology 98:S27

Measurable and science-based citrus propagation risk mitigations ensure that quarantine pests are not imported into citrus producing areas. The National Plant Protection Organization (NPPO) of each country safeguards the movement of citrus propagative material. Pest Risk Analysis conducted under International Plant Protection Convention (IPPC) guidelines assesses specific phytosanitary hazards and identifies mitigations for managers of risk. NPPOs develop models to decide which mitigations should be used. A Hazard Analysis and Critical Control-point (HACCP) based model is a tool to provide guidance to NPPOs when developing measurable control systems for phytosanitary hazards. HACCP models were produced from the FAO/IBPGR Technical Guidelines for the Safe Movement of Citrus Germplasm (FAO, 1991), the Citrus Nursery Stock Certification Manual Procedure Manual (DPI, 2006), and the University of California Citrus Clonal Protection Program (UCRiverside, 2007) which are existing guidelines used for risk mitigation. A flow diagram for critical control points (CCP) in each system was constructed. All phytosanitary risks underwent a hazard analysis and mitigations were identified. Most importantly a critical limit was recommended for each CCP identified. This led to the establishment of a monitoring system for each CCP in each system. A model system for use by NPPOs to measure citrus propagation mitigations was developed.

Responses of almond trees and rhizosphere fungi to novel pre-plant soil fumigation treatments for control of Prunus replant disease

G. T. BROWNE (3), L. S. Schmidt (3), B. A. Holtz (1), D. A. Doll (2), S. K. Upadhyaya (4), B. D. Lampinen (4)
(1) UCCE, Madera, CA, USA; (2) UCCE, Merced, CA, USA; (3) USDA-ARS, Davis, CA, USA; (4) University of California, Davis, CA, USA
Phytopathology 98:S27

Prunus replant disease (PRD) suppresses growth of young almond and stone fruit trees in soil recently devoted to them. Pre-plant soil fumigation, especially with chloropicrin (CP), can prevent PRD, but in California there is a legislative mandate to reduce emissions of fumigants into the atmosphere. To address this and examine PRD etiology, we applied 12 pre-plant soil fumigation treatments with tractor-mounted shanks in fall 2006 on land previously devoted to almonds near Firebaugh, CA; included were different rate and spatial treatments with CP and mixtures of it with 1,3-dichloropropene (1,3-D), and iodomethane (IM); 1,3-D; and methyl bromide (MB). Conventional fumigant metering was used for strip and broadcast spatial applications; GPS-controlled metering was used for spot applications. All plots were planted with almond on Nemaguard rootstock Jan 2007, and resulting trunk diameters were measured Feb 2008. Roots were sampled from healthy and PRD-affected trees in Jun and Aug 2007 and subjected to culture-dependent and culture-independent microbial examinations. All treatments with CP or mixtures of it with 1,3-D or IM (spot, strip, and broadcast treatments; rates 224 to 627 kg/treated ha, depending on fumigant) prevented PRD (trunk diameters 70 to 101% greater than the control), but MB and 1,3-D were less effective (trunk diameters 24 to 27% greater than the control). Redundancy analysis linked root-associated *Cylindrocarpon* spp., *Fusarium* spp., and additional fungi to PRD-affected trees and *Trichoderma* spp. to healthy trees. Use of small, spatially focused doses of fumigants can prevent PRD and reduce emissions, and fungal community shifts are associated with the disease.

Velvetleaf sensitivity to IAA and rhizobacteria that produce IAA-like compounds

H. Brubaker (1), R. ZDOR (1)
(1) Andrews University
Phytopathology 98:S27

The use of IAA-producing rhizobacteria in weed control may prove to be an useful tool in integrated weed management. This project examined the sensitivity of the weed velvetleaf to purified IAA and to rhizobacteria that produce IAA-like compounds. IAA levels greater than 1 micromolar inhibited root growth *in vitro*. Rhizobacterial isolates recovered over a 3 year period from velvetleaf root surfaces and interiors that produce IAA-like compounds were tested for their ability to inhibit velvetleaf plant growth. Nine isolates were included in the analysis (7 *Rhizobium radiobacter*, 1 *Rhizobium rubi*, and 1 *Serratia marcescens*). Tryptophan, a precursor for IAA synthesis, was found to stimulate production of IAA-like compounds (as determined using Salkowski reagent). Each isolate was used to inoculate velvetleaf seeds in sterilized soil with and without low amounts of tryptophan. Inoculated plants irrigated with tryptophan-containing water consistently had reduced growth and survival relative to plants lacking tryptophan exposure. These results are consistent with the hypothesis that IAA-like compounds are involved in mediating plant suppressive effects of certain rhizobacteria on velvetleaf growth.

Leaf spot on Tigergrass caused by *Exserohilum rostratum* in Florida

A. M. BRUNINGS (1), L. E. Datnoff (1), A. J. Palmateer (4), J. C. Locke (2), J. M. Frantz (2), C. R. Krause (3)
(1) Department of Plant Pathology, Gainesville, FL, USA; (2) USDA-ARS, Toledo, OH, USA; (3) USDA-ARS, Wooster, OH, USA; (4) University of Florida, Homestead, FL, USA
Phytopathology 98:S27

Tigergrass (*Thysanolaena maxima*) is a commercial containerized and landscape ornamental grass from the family Poaceae similar in appearance to bamboo. In the summer of 2006, a leaf spot was first noticed in a South Florida nursery, and the disease has since then been observed in several nurseries and landscapes throughout Miami-Dade county and the Florida Keys. In addition, leaf spots have been observed on young transplants from production greenhouses in Apopka, FL. Symptoms start as minute tan colored flecks often turning chlorotic to necrotic that elongate elliptically between the leaf veins, sometimes with a yellow halo, eventually turning all necrotic. Individual lesions will coalesce into large necrotic elliptical spots to blotches, sometimes interspersed with chlorosis. Infected leaf tips may turn light brown to brown, curl and turn yellow away from the leaf tip. *Exserohilum rostratum* was consistently isolated from the lesions incubated in moist chambers, and identified based on conidial morphology and ITS1/ITS4 sequencing. Pathogenicity was confirmed by spraying conidia from 7-day old cultures at inoculum densities varying from 10^3 to 10^5 conidia/ml with a surfactant (Tween20) onto tigergrass plants. Plants were covered with plastic bags overnight and maintained at 25°C. Symptoms appeared as early as 18 hours post inoculation, while control plants sprayed with water and Tween20 did not develop symptoms. This appears to be the first report of *E. rostratum* infecting *T. maxima* in Florida.

Transcriptome analysis of the silicon-*Magnaporthe grisea* interaction

A. M. BRUNINGS (1), L. E. Datnoff (1), J. F. Ma (4), N. Mitani (3), Y. Nagamura (3), B. Rathinasabapathi (2)
(1) Department of Plant Pathology, Gainesville, FL, USA; (2) Horticulture Dept., UF, Gainesville, FL; (3) National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; (4) Research Institute for Bioresources, Okayama University, Kurashiki, Japan
Phytopathology 98:S28

Silicon is known to increase resistance to the rice blast pathogen *Magnaporthe grisea*. To investigate the extent to which the transcription profile of rice is altered in plants amended with silicon in response to *M. grisea*, a microarray study with a 44-kb genechip was conducted to assess the global activation/repression of rice genes using the rice cultivar Monko-to and *M. grisea* 86-137. Plant gene expression was analyzed by comparing plants amended with silicon to untreated control plants (Si/c), plants inoculated with the pathogen compared to those untreated (P/c), plants amended with silicon and inoculated with the pathogen compared to plants amended with silicon (SiP/Si), and plants amended with silicon and inoculated with the pathogen compared to plants inoculated with the pathogen (SiP/P). The Si/c comparison yielded 281 differentially regulated genes, including metabolic and plant defense response genes. Not surprisingly, 486 genes were differentially regulated in the P/c comparison. Most interestingly, 432 genes were differentially regulated in the SiP/P comparison, of which 231 were unique for this comparison. In the SiP/P comparison, 491 transcripts were differentially regulated, of which 167 were unique. In addition to known pathogenicity response genes, a number of genes encoding transcription factors, and proteins with protein-protein interaction domains, were identified as unique to the response of Si-amended rice to the blast pathogen. The data suggest that Si-mediated resistance involves multiple pathways, changing the plant response to rice blast infection. Silicon amendment clearly results in altered plant defense signaling, rendering the plant more resistant to infection by *M. grisea*.

Changes in soybean proteome associated with growth promotion by seed treatment with *Bacillus amyloliquefaciens* KPS46

N. BUENSANTEAI (1), S. Prathuangwong (1), G. Y. Yuen (2)
(1) Kasetsart University, Chatuchack, Bangkok, Thailand; (2) University of Nebraska - Lincoln, Lincoln, NE, USA
Phytopathology 98:S28

Bacillus amyloliquefaciens KPS46 is a rhizobacterium that induces systemic resistance and enhances plant growth in soybean. It was unknown if it promotes plant growth by controlling plant pathogens or directly affecting plant growth processes. The objective of this study was to test the hypothesis that KPS46 stimulates production of plant proteins involved in metabolism and growth. Soybean seed treated with KPS46 or with water were germinated in plates of agar media. After incubation for 14 days, KPS46 treated seedlings had longer roots and greater biomass than the water control. Hypocotyl tissues from the two treatments were harvested for protein extraction. Following separation by 2-D polyacrylamide gel electrophoresis, 20 proteins up-regulated by KPS46 were analyzed by mass spectrometry to determine their amino acid sequences. The proteins fell into several functional categories, several of which are related to growth and development. They included metabolism (glutamine synthetase precursor); energy/ pentose phosphate (rubisco subunit and ribulose-bisphosphate carboxylase); energy/glycolysis/ glycoxylase cycle/ gluconeogenesis (glyceraldehyde-3-phosphate dehydrogenase and malate synthase); defense and stress response (catalase, lectin, and probable gamma-glutamyl hydrolase), protein destination and storage (vegetative storage protein precursor, beta-conglycinin beta subunit and alpha subunit of beta conglycinin); and carbohydrate metabolism (soybean agglutinin). This demonstrated that KPS46 can enhance plant growth under gnotobiotic conditions by direct stimulation of plant protein production.

Chemical growth inhibition as phenotypic markers for matching isolates of *Pythium* within species

M. BURGOS (1), G. W. Moorman (1)
(1) Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802
Phytopathology 98:S28

The ability to match Pythium isolates causing crop losses with those from various locations in the production system would facilitate identifying pathogen harbors. Although microsatellite genotypes have been developed for Pythium aphanidermatum, P. irregulare, and P. cryptoirregulare, additional markers are needed to confirm matches among isolates. The objective of this study was to assess growth inhibition phenotypes for matching isolates of Pythium when grown on agar amended with malachite green (5 µg/ml), sodium chloride (0.25 M), dimethomorph (100 µg/ml), rose bengal (200 µg/ml), zinc sulfate (100 Zn µg/ml), copper sulfate (5 Cu µg/ml), or fenamidone (5 µg/ml). Variation in growth inhibition found among isolates may be combined with genotype to match isolates.

Tissue-specific colonization of sorghum caryopses by grain mold fungi

D. N. Butler (1), L. W. Noll (1), C. R. LITTLE (1)
(1) Kansas State University, Manhattan, KS, USA
Phytopathology 98:S28

In temperate production regions, grain mold susceptible (GMS) and resistant (GMR) sorghum genotypes are colonized by *Fusarium* spp. and *Alternaria alternata*. The most damaging infections occur at anthesis, colonize the lodicule and ovary base and then progress in an acropetal fashion as the caryopsis matures. Caryopses were dissected into four tissues (black layer, pericarp, endosperm, and germ), incubated in dsH_2O or 0.05% NaOCl, and plated on half-strength PDA to isolate total fungi. In all cases, black layer yielded the highest levels of total fungi, total *Fusarium* spp., *A. alternata*, as well as other saprophytes and weathering fungi (*Aspergillus* spp., *Cladosporium* spp., *Epicoccum* spp., and *Penicillium* spp.). Endosperm and germ yielded significantly lower ($P < 0.05$) levels of GM fungi compared to black layer or pericarp. *Fusarium* spp. isolated from caryopsis tissues were identified to species using morphology and PCR. Isolation of *Fusarium* spp., including *F. thapsinum* (FT), was significantly greater ($P < 0.05$) from GMS versus GMR germ. In general, isolation of fungi from black layer and pericarp was not significantly different for GMS and GMR genotypes. This study indicated that the majority of fungi reside in/on the black layer and surrounding pericarp. Colonization of germ and endosperm tissues by GM fungi, especially FT, served as the best indicator for GM resistance or susceptibility when screening sorghum seed.

Evaluating tetrazolium staining of sorghum caryopses as a screen for grain mold resistance

D. N. Butler (1), L. W. Noll (1), C. R. LITTLE (1)
(1) Kansas State University, Manhattan, KS, USA
Phytopathology 98:S28

For sorghum, tetrazolium (TZ) staining is not always a useful indicator for stand establishment and vigor. However, little work has been done to examine the efficacy of TZ in screening sorghum seed for grain mold resistance (GMR). In this study, seed from six genotypes (Sureno, BTx631, BTx623, RTx430, RTx2911, and SC170) were collected, bisected, and treated with TZ dye. Assessments of viable (+) or non-viable (-) seed were obtained from sets of kernel halves in each sample and the other sets were used for isolation of fungi. Viability (+) was based upon complete staining of radicle, embryo axis, plumule, and coleoptile, > two-thirds staining of the scutellum, and absence of mechanical damage to the embryo as described by the Association of Official Seed Analysts. Sureno (GMR) exhibited significantly higher ($P < 0.05$) seed TZ viability than the other genotypes. Regression of % fungal isolation upon seed viability resulted in significant negative correlations ($P < 0.05$) between TZ viability and isolation of grain mold and weathering fungi from caryopses. These included *Fusarium thapsinum* (FT), *Alternaria alternata*, *Cladosporium cladosporoides*, *Penicillium* spp., and *Epicoccum nigrum*. Additionally, TZ viability was positively correlated with isolation of *Fusarium* spp. (other than FT) and *Aspergillus niger*. Thus, TZ reaction in sorghum caryopses appears to be a potential indicator of fungal colonization and GMR for the genotypes tested in this study.

Integrating GPS, GIS and geostatistics for risk assessment of *Bean pod mottle virus* in Iowa

E. BYAMUKAMA (1), A. Robertson (1), F. W. Nutter, Jr. (1)
(1) Iowa State University
Phytopathology 98:S28

Use of Global Positioning Systems (GPS), Geographic Information Systems (GIS), and geostatistics are becoming powerful tools in visualizing, planning, and developing decision support models to improve the management of plant pathogens. These tools were used in mapping the prevalence and incidence of *bean pod mottle virus* (BPMV) of soybean in Iowa for three growing seasons (2005–2007). The GPS coordinates for each soybean field sampled (8-20/county) were recorded prior to sampling and 30 plants/soybean field were sampled and tested for the presence of BPMV by ELISA. Field and county-level BPMV prevalence and incidence were mapped using ArcGIS. Both BPMV prevalence and incidence data at the field and county level varied among the three years, with 2006 having the highest prevalence of BPMV-infected fields and counties. County-level prevalence of BPMV was 39/96 counties in 2005 (40.6%), 90/99 counties (90.1%) in 2006, and 74/99 counties (74.7%) in 2007. The incidence of BPMV within counties ranged from 0 to 100%. At the county level, there was significant spatial dependence for BPMV incidence based on Moran's I analysis, indicating that counties with high BPMV incidence tended to be neighbored by counties that also had high BPMV incidence, and counties with low BPMV incidence tended to be neighbored by counties with low BPMV incidence. Krigged maps for the three years revealed greater BPMV incidence in the southern half of the state compared to the northern half. Higher incidence of BPMV in the southern Iowa was linked to high winter survival of bean leaf beetle (*Ceretoma*

trifurcata) populations - the major vector of BPMV. The use of GPS, GIS, and geostatistics greatly improved our understanding of the geographical distribution of BPMV in Iowa and which led to the development of a conceptual model for the county-level risk factors that influence BPMV occurrence.

Within-field spatial and temporal analysis of *Bean pod mottle virus* in Iowa

E. BYAMUKAMA (1), A. Robertson (1), D. Nordman (1), F. W. Nutter Jr. (1)
(1) Iowa State University
Phytopathology 98:S29

To quantify the temporal and spatial spread of *bean pod mottle virus* (BPMV), a major pathogen of soybean (*Glycine max*) in the U.S., a quadrat-based method was developed. In 2006 and 2007, 30-cm-long quadrats were established within field plots (6 rows wide × 7.5 m long with 25 quadrats per row = 150 quadrats per plot). Quadrats were thinned to four soybean plants/quadrat. Four treatments were used in an attempt to affect the temporal rate of BPMV epidemics in soybean. Treatments were (i) establishing a BPMV inoculum source (2 quadrats inoculated), (ii) two foliar insecticide applications (at V1 and R2 growth stages), (iii) BPMV-inoculated and two foliar insecticide applications, and (iv) a non treated control. Quadrats were sampled by selecting the youngest, fully-expanded leaflet from each of four plants within a quadrat. Sampling began 25 days after planting and continued at 8–11 day intervals until crop senescence. Leaf sap was extracted from each 4-leaflet (bulked) sample (from each quadrat), and tested for presence of the BPMV by ELISA (Agdia, Elkhart, IN). Quadrat position (plot, row, and quadrat number), and the date of sampling that each quadrat first tested positive for BPMV were recorded and mapped. *Bean pod mottle virus* was detected as early as the first sampling date (30 May 2006 and 12 June in 2007). The rate of BPMV infection in 2006 ranged from 0.09 to 0.17 *logits*/day, indicating that BPMV incidence was doubling every 4.1 to 7.7 days. Doubling times in 2007 were slower, ranging from 17.3 to 34.7 days. Plots that had the earliest onset of BPMV also had the highest BPMV incidence at the end of the growing season (R^2 = 76.9%). Spatial analyses using both ordinary runs and black and white joins revealed clustering of BPMV-infected quadrats occurred throughout the growing season.

Evaluation of transgenic *Ophiosphaerella herpotricha* expressing green and red fluorescent proteins in turf-type bermudagrass

O. C. CAASI (2), N. R. Walker (2), S. M. Marek (2), T. K. Mitchell (1)
(1) Ohio State University, Columbus, OH, USA; (2) Oklahoma State University, Stillwater, OK, USA
Phytopathology 98:S29

Ophiosphaerella herpotricha is one of the causal agents of spring dead spot in turf-type bermudagrass (*Cynodon* spp. and interspecific hybrids) in the transition zone of the United States. Isolates of *O. herpotricha* were transformed using *Agrobacterium tumefaciens* to express either green (GFP) or red (tdTomato) fluorescent protein. Two turf-type cultivars, Tifway 419 and Midlawn and a *Cynodon transvaalensis* accession were used to study the infection and colonization of roots by *O. herpotricha* under controlled conditions. Roots were inoculated with fungal mycelium and plants were incubated at 17°C. At 2 DPI (days post-inoculation), transverse and longitudinal sections revealed fungal penetration through intact root epidermal layers for all plants. At 10 DPI, *O. herpotricha* colonized root cortical tissues of Tifway 419 and Midlawn intracellularly, formed aggregated hyphae, and caused necrotic lesions and collapse of cortical cells. At 14 DPI, roots of Midlawn and Tifway 419 exhibited a range of necroses while roots of *C. transvaalensis* were much less necrotic and appeared tolerant to infection by *O. herpotricha*.

***Gazania* new host of *Oidiopsis taurica* in Corrientes and Resistencia gardens, in Argentina**

M. G. Cabrera (1), R. E. Alvarez (1), M. A. RYBAK (2)
(1) Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Argentina; (2) INTA, Bella Vista, Corrientes, Argentina
Phytopathology 98:S29

Gazania × hybrid, Asteraceae, is an exotic plant widely used as ornamental. Since 2004, in several gardens from Corrientes and Resistencia cities, in Argentina, young plants of Gazania affected with a powdery mildew was found. Plants showed characteristic chlorosis and irregularly shaped lesions on adaxial leaves surface. The fungus covered the lower leaf surface. The objective of this study was to determine the causal agent of disease. Epidermal strip from lower surface of leaves and leaf cross sections were mounted in water and examined with a light microscope. These preparations showed that conidiophores developed from endophytic mycelium, and emerged through stomata. Long conidiophores hyalines, septate, single or branched, mostly about more that 100 µ, were observed. They usually carried one conidium. Hyaline single-cell conidia were dimorphic. Primary conidia were clavate, acuminate towards the tip, and measured 60 × 17.4 µ. The secondary conidia were obpyriform and measured 55 × 17 µ. On these anamorphic characters (Oidiopsis) the pathogen was identified as Leveillula taurica Lév. Arnaud. The powdery mildew on gazania plants reduced the quality of appearance of this ornamental specie. Gazania × hybrid result a new host of L. taurica in Corrientes and Resistencia cities of Argentina.

Biological control of *Gibberella zeae* with *Trichoderma* spp.

M. Cabrera (1), S. A. PEREYRA (2), S. Vero (1)
(1) Fac. de Quimica, UDELAR; (2) INIA - Natl Inst for Agric Research, Colonia, Uruguay
Phytopathology 98:S29

Fusarium head blight (FHB), primarily incited by *Fusarium graminearum* (*G. zeae*) is a destructive disease of wheat and barley in Uruguay. This study evaluated the effectiveness of *Trichoderma* spp. in reducing perithecial production of *G. zeae* on wheat residue. Sixteen *Trichoderma* isolates obtained from wheat residue were identified to species based on morphology and molecular techniques using sequencing region ITS1-ITS2 and GENBANK comparison. Isolates were identified as *T. longibrachiatum*, *T. atroviride*, *T. koningiopsis* and *T. harzianum* and later characterized for production of chitinases, beta 1-3glucanases, proteases, xylanases, antifungal (volatile and diffusible) compounds, and antagonistic activity measured as growth inhibition in dual cultures and reduction of *G. zeae* perithecial production. A *G. zeae* isolate with high DON production, aggressiveness and perithecial production was selected to be used in this study. No significant differences were found among isolates for lytic enzymes production. The most efficient isolates inhibiting *G. zeae* growth in dual cultures were TR7B (*T. koningiopsis*), TmE (*T. atroviride*), TmB (*T. atroviride*) and To (*T. atroviride*). Moreover, isolate TmE produced the greatest *G. zeae* growth inhibition by volatile and diffusible compounds. Based on the information of production of antifungal compounds and dual cultures, five isolates were selected to determine their effect on the production of *G. zeae* perithecia on wheat residue. Spore suspensions of *Trichoderma* isolates were coinoculated with *G. zeae* on wheat residue. To facilitate perithecia development, residue was incubated at 20–22°C under 12-hr UVlight and dark cycles for 90 days. Isolates TmE and T1c (*T. koningiopsis*) significantly reduced perithecia production. Further research is needed on the mechanisms involved in *G. zeae* perithecial reduction by *T. atroviride and T. koningiopsis*.

Functional and structural characterization of cerato-platanin proteins in *Moniliophthora perniciosa*, the cause of Witches' Broom disease in cacao

O. G. CABRERA (1), G. Zaparoli (1), F. J. Medrano (1), R. A. Tiburcio (1), G. G. Lacerda (1), G. G. Pereira (1)
(1) UNICAMP, Campinas, São Paulo, Brazil
Phytopathology 98:S29

Witches' Broom disease (WBD) of cacao is one of the most important phytopathological problems in South America. The causal agent of WBD is the fungus *Moniliophthora perniciosa* whose genome project we started in 2001. We did find sequences coding for putative proteins belonging to two classes of necrosis inducing factors: 3 genes belonging to Necrosis and Ethylene Inducing Proteins (MpNEP) and 5 genes similar to cerato-platanins (MpCP). The aim of this work is to understand the role of MpCPs in the pathogenicity of *M. perniciosa* comparing with MpNEPs activity, already described. MpCP1 was expressed *in vitro* and caused necrosis in tobacco and cacao leaves. MpCP1 and MpNEP2 produced different necrosis profiles suggesting differences in their mechanisms of action. Mixture of MpCP1 with MpNEP2 led to a synergistic necrosis effect in tobacco leaves. Transcription analysis showed that MpCP1 and MpNEP2 are expressed even before the beginning of the necrosis signal. MpCP1 and MpNEP2 were able to induce necrosis after thermal treatment showing that they are stable. Small Angle X-ray Scattering measurements suggested that MpCP1 and MpNEP2 were dimers in solution and allowed the construction of a low resolution model of the envelope of each protein. This is the first report of a member of Basidiomycota presenting both NLPs and CPs suggests that this might be relevant for the progress of the disease and turning them in potential candidates to develop control strategies.

Seventy years of screening for resistance to grape downy mildew – without consensus

L. CADLE-DAVIDSON (1)
(1) USDA-ARS, Grape Genetics Research Unit, Geneva, NY 14456, USA
Phytopathology 98:S29

Over the past 70 years, at least ten screens for resistance to grape downy mildew have been conducted around the world using uncharacterized populations of the pathogen *Plasmopara viticola*. Summarizing the results into three categories (resistant, moderate, susceptible) for shared host genotypes, only 32% of the ratings corresponded, due to variable environment, pathogen source, or mislabeling of host genotype. To establish a baseline for controlled

inoculation studies, a single isolate of *P. viticola* was inoculated in 2006 onto 883 genotypes of wild *Vitis* spp. and named cultivars. A second isolate was used in 2007, and ratings were consistent for 95% of genotypes across the two years. For the 89 shared host genotypes screened in this and previous studies, only 37% of the ratings corresponded. Resistant and susceptible genotypes were identified in every well-represented species except *V. vinifera* and *V. acerifolia*, which had only susceptible genotypes. Of the varieties previously screened in the U.S., only *V. hybrid* cvs. 'Yates' and 'Concord' were resistant or moderately resistant at all locations including this study. Grape breeders should test germplasm for downy mildew resistance in diverse environments to know the spectrum of resistance efficacy. Future screens should consider intraspecies variation in resistance, report identities for wild and cultivated genotypes, and carefully consider the genetics of the pathogen.

Comparative virulence of Rhizoctonia spp. pathogenic to Lepidium draba assessed using survival analysis

A. CAESAR (1), T. Caesar (1)
(1) USDA/ARS Northern Plains Agricultural Research Laboratory
Phytopathology 98:S30

Isolation of fungi from insect-damaged roots of the invasive perennial Lepidium draba surveyed over 1999–2007 in Europe revealed that such roots of this species were often infected with one or more soilborne fungi. Plants with evident stunting and/or chlorosis and reddening of leaves nearly always exhibited root damage by one or more insect species. More than half of the plants with such symptoms were found to have galls typically caused by Ceutorhynchus spp. larvae. Throughout the surveyed range of L. draba, the most prominent fungus isolated from root galls and adjacent root tissue was Rhizoctonia solani. The galls were often decayed at exit points of the larvae and adjacent root tissue was consistently infected with Rhizoctonia solani, Fusarium and Pythium spp. Eight of 11 isolates of Rhizoctonia solani anastomosed with tester isolates of AG-4, one was a binucleate and two isolates were unidentified to-date. Five isolates caused mortality of 9–10 plants when 5-wk-old seedlings were planted in soil mix infested with Rhizoctonia inoculum. In previous studies, a similar complex of soilborne fungi had been found in association with insect root herbivory of Euphorbia esula/virgata, Centaurea maculosa and Centaurea diffusa. The prevalence of such associations, especially in yet another instance involving a highly invasive herbaceous perennial weed may indicate that such highly destructive insect/pathogen combinations are a previously overlooked key to biological control success. A concept for utilizing a multitrophic approach to screening for new biocontrol agents of exotic, invasive herbaceous perennial weeds of rangelands will be discussed. A novel approach to assess comparative virulence of the isolates was to use parametric survival models for the data, which was interval censored. A logistic model most clearly indicated the differing rates of mortality caused by the R. solani isolates. Such a procedure could be used in similar cases.

Fungal diseases on *Vaccinium meridionale* in Colombia

C. Calderon (1), C. Socha (1), P. JIMENEZ (1)
(1) Universidad Militar Nueva Granada, Bogota D.C., Colombia
Phytopathology 98:S30

Vaccinium meridionale is a member of the family Ericaceae that occurs in Colombia Ecuador and Venezuela, above the 2400 m elevation. Its berries have high antioxidant content, making them a good option for the berries market. Even though this species remains undomesticated, and the fruits are collected from natural occurring bushes, its fruits are very appreciated in the local market and efforts directed to export them have started. In Colombia, at least, domestication efforts are just starting and information about the pathogens affecting the crop are necessary. During 2006, three localities of Cundinamarca state were visited and leaves showing lesions were sampled. From this lesions fungi from the genera *Pestalotiopsis*, *Pestalotia*, *Epicoccum*, and *Alternaria*, were isolated. Since this species takes a long time for growing, Koch's postulates were completed on detached leaves, corroborating the pahogenicity of these fungi.

Genomic analysis of soybean defense response to *Sclerotinia sclerotiorum*

B. CALLA (3), D. Simmonds (1), S. J. Clough (2)
(1) Agriculture and Agri-Food Ottawa, Ontario, Canada; (2) USDA-ARS and the University of Illinois, Urbana, IL, USA; (3) University of Illinois, Urbana, IL, USA
Phytopathology 98:S30

We have conducted microarray studies on changes in soybean transcript levels in response to *Sclerotinia sclerotiorum* infection. These stem inoculations enabled the identification of genes that are significantly differentially expressed in soybean plants in partially resistant versus susceptible varieties. We are expanding these studies to include effects of oxalic acid (OA), a major virulence factor *S. sclerotiorum*. The OA studies involve leaf inoculation or OA infiltration in an oxalate-oxidase resistant transgenic, and its susceptible, parent. To assist with the identification of key defense genes, we assigned genes into functional categories based on the annotation of their closest sequence match in public databases and we clustered the genes across multiple experiments. Candidate defense genes are being further characterized by quantitative real-time RT-PCR to verify that the correct gene was identified in the microarray experiments. Promising genes will be functionally characterized by obtaining knockouts of these genes in soybean and *Arabidopsis thaliana*. To obtain knockouts in soybean we will use a viral induced gene silencing system and/or generate stable transgenics utilizing RNAi constructs. Additionally, overexpression of candidate defense genes will be studied in Arabidopsis and soybean. Promising candidate defense genes will be mapped to see if they map to known QTLs related to defense to *S. sclerotiorum*.

Efficacy of *Muscodor albus* for control of Phytophthora blight on bell pepper and butternut squash in the greenhouse

A. R. CAMP (1), H. W. Lange (1), H. R. Dillard (1), C. D. Smart (1)
(1) Cornell University NYSAES, Geneva, NY, USA
Phytopathology 98:S30

The soil-borne oomycete *Phytophthora capsici* causes Phytophthora blight on cucurbits and peppers, resulting in serious losses to vegetable growers. Fungicides and crop rotation do not always prevent significant yield losses. It has been reported that the fungus *Muscodor albus* can be used as a soil biofumigant to control Phytophthora blight, and might be an alternative to chemical fungicides. *P. capsici*-infested potting mix was inoculated with three rates of *M. albus*, mefenoxam (Ridomil Gold EC, Syngenta Crop Protection, Inc.) or no treatment. Seedlings of five bell pepper cultivars (with varying levels of tolerance to *P. capsici*) and one butternut squash cultivar were transplanted into the treated potting mix. After 7 days in the greenhouse under natural light conditions, the plants were rated on a scale of 0 (healthy) to 5 (dead). The experiment was performed 3 times. There was a significant interaction between pepper cultivar and soil treatment, and mefenoxam reduced disease severity on all cultivars (pepper and squash). On the more tolerant pepper cultivars, disease severity was only slightly lower on plants treated with the highest rate of *M. albus*, compared to the pathogen-only control, while no significant decreases were observed with butternut squash or the susceptible pepper cultivar Red Knight. These results indicate that, as applied in this study, *M. albus* does not control *P. capsici* on tolerant or susceptible bell peppers or butternut squash.

Impact of crop rotation on the occurrence of diseases and nematodes in corn, cotton, and peanut in southwest Alabama

H. L. CAMPBELL (1), A. K. Hagan (1), K. L. Bowen (1), M. D. Pegues (2)
(1) Auburn University, Auburn, AL; (2) Gulf Coast Research and Extension Center, Fairhope, AL
Phytopathology 98:S30

A study was begun in 2002 at the Gulf Coast Research and Extension Center, Fairhope, AL to assess the impact of crop rotation on diseases of peanut and corn as well as nematode population in corn, cotton, and peanut. Peanuts were rated for early and late leaf spot, stem rot, and TSWV. Soil samples were taken for nematode assays shortly after each crop was harvested. In, 2006 and 2007, peanut rotation sequence had no significant effect on leaf spot severity, peanut rust, and stem rot incidence. Incidence of TSWV was influenced by peanut cropping frequency in 2007 but not 2006. Foliar disease incidence in corn, including plots kept in continuous corn for five years was negligible. Yield for peanut following 1 yr of corn was significantly lowered compared with peanuts cropped behind three yrs corn. Among the corn rotations, root knot nematode larvae counts were higher on continuous corn. Increases in root knot nematode populations may be affecting corn yields. Declining cotton yields seen in 2007 may also be associated with a increase in root knot larvae counts.

Occurrence of copper resistance in *Xanthomonas axonopodis* pv. *citri* in Argentina

B. I. Canteros (1), M. RYBAK (1), A. Gochez (1), P. Velazquez (3), M. Rivadeneira (5), M. Mitidieri (4), S. Garran (2), L. Zequeira (1)
(1) INTA, EEA Bella Vista, Ctes., Argentina; (2) INTA, EEA Concordia, Entre Rios, Argentina; (3) INTA, EEA Famailla, Tucuman, Argentina; (4) INTA, EEA San Pedro, Buenos Aires, Argentina; (5) INTA, EEA Yuto, Jujuy, Argentina
Phytopathology 98:S30

Copper resistance was detected for the first time in Argentina in 1994 after decades of citrus canker control with copper-containing bactericides. The addition of mancozeb to the copper products was recommended immediatelly. Since 1994 and till March 2008 we surveyed all citrus growing areas of Argentina taking samples of canker-affected tissue and testing the copper susceptibility of the isolated strains. Cu-resistant strains were those that grew on lima bean agar plus 200 ppm of copper sulfate and in water suspensions of

bactericides containing 1.5 grams metallic copper per liter during 36 hours. Cu-susceptible strains died after 30 minutes in water plus Cu and grew in lima bean agar without copper and did not grow in solid medium with copper. A total of 3350 strains were rated, about 10% of them were Cu-R. The resistant strains were only from several groves in the NW of Corrientes Province from 1994, from one grove in Formosa in 2004, and from one grove in the SE of Corrientes in 2007. All the other strains from other groves located in Corrientes, Entre Ríos, Misiones, Formosa, Chaco, Buenos Aires, Tucumán, Salta, Jujuy, and Catamarca provinces were susceptible to copper containing bactericides. The early application of the mix of mancozeb to copper at least once a year was enough to stop or delay the occurrence of Cu resistant strains.

Molecular identification of pathotypes of *Plasmodiophora brassicae*, causal agent of clubroot of crucifers, from Canada

T. CAO (1), S. Xue (1), S. E. Strelkov (1)
(1) University of Alberta, Edmonton, AB, Canada
Phytopathology 98:S31

The protist, *Plasmodiophora brassicae*, is a soilborne pathogen that causes clubroot, an economically important disease of canola and other cruciferous crops. Populations of *P. brassicae* may consist of different pathotypes, complicating efforts to develop clubroot-resistant host genotypes. Conventional bioassays to classify isolates into pathotypes are labor intensive, time consuming and costly. Thus, cleaved amplified polymorphic sequence (CAPS) markers were developed to distinguish between two of the major *P. brassicae* pathotypes found in Canada. The *P. brassicae*-specific primers PbsF1/R1 and PbsF2/R2 were designed based on a small subunit ribosomal RNA gene sequence from the pathogen. Both sets of primers amplified a PCR product about 2 kb in size from *P. brassicae*-infected root tissue. Digestion of the amplicon with the restriction enzymes *Mlu*I and *Pvu*II yielded two major bands from tissues infected with isolates representing pathotype P3, whereas three major bands were obtained from populations representing pathotype P2, as designated on the differentials of Somé et al. This suggests that CAPS analysis could be used as a quick and reliable tool for routine identification of *P. brassicae* pathotypes. The amplicons obtained are currently being sequenced to enable comparisons at the DNA level.

Phylogeny, function and structure of rice oxalate oxidases

M. C. Carrillo (3), P. H. Goodwin (2), J. E. Leach (1), H. Leung (3), C. M. VERA CRUZ (3)
(1) Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80537-1177; (2) Department of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G2W1; (3) International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines
Phytopathology 98:S31

Oxalate oxidases (*OxO*) in cereals have been implicated to play a role in defense response to pathogen infection. Here, we analyzed the molecular changes in members of rice *OxO* (*OsOxO*) genes mapped to chromosome 3 associated with resistance to blast. *OxOs* are members of the cupin proteins with the conserved motifs ([G(X)5HXH-(X)3,4E(X)6G] and G(X)5PXG(X)-2H(X)3N]) forming the active sites of the enzyme. There are four tandemly duplicated rice oxalate oxidases (*OsOxO*) exhibiting >90% similarity at the nucleotide and amino acid level. Expression analysis using resistant and susceptible advance backcross lines of Vandana/Moroberekan showed that only *OsOxO4* is expressed during rice-*Magnaporthe oryzae* interaction. Expression data from the Rice Gene Indices and Rice MPSS databases show that *OsOxO1* is expressed at the flowering stage, *OsOxO3* is in roots and *OsOxO4* is in leaves and expressed during biotic and abiotic stress conditions. No expression evidence was found for *OsOxO2*. Analyses of the overall *OsOxO* sequence from 62 rice cultivars show that purifying selection is the major factor maintaining *OxO* protein homogeneity although a 134-bp conserved region in *OsOxO4* were under diversifying selection. Phylogeny of the 62 cultivars using the *OsOxO* sequences show that majority of the cultivars fall into the expected subgroups – indica, aus, tropical japonica and temperate japonica. Characterization of SNPs and InDels in each gene across cultivars using PolyPhen and SIFT analyses tools identified sites that are of functional importance. Plotting the SNPs against the different conserved domains of *OsOxO* shows that these are highly conserved, and SNPs found in these regions were either silent mutations or led to tolerated amino acid changes. No SNPs or InDels were found in motif 16, a unique motif in cereal *OxOs*. Overall, our data suggest *OxO* in rice are conserved among different cultivars suggesting an important role in diverse biotic stress and developmental pathways.

***Avena barbata*, a potential source of new crown rust resistance in oat**

M. L. CARSON (1)
(1) USDA-ARS Cereal Disease Lab, St. Paul, MN, USA
Phytopathology 98:S31

The use of race-specific seedling genes for resistance has been the primary means of controlling crown rust of oat. As resistance genes from hexaploid cultivated oat, *Avena sativa* and later, the wild hexaploid animated oat, *A. sterilis* were deployed in oat cultivars, corresponding virulence in the crown rust population increased rapidly, such that the effective lifespan of a resistant cultivar is now five years or less. Introgression of resistance genes from diploid and tetraploid *Avena* species into hexaploid oat has been difficult due to differences in ploidy levels and the lack of homology of chromosomes between the two species. The wild tetraploid oat, *A. barbata*, has been a source of powdery mildew and stem rust resistance in oat, but has largely been unexploited for crown rust resistance. A total of 359 accessions of *A. barbata* from the National Small Grains Collection were evaluated in seedling greenhouse tests. Thirty-nine percent of accessions were at least moderately resistant when inoculated with a crown rust race with low virulence (DBBC). When tested further with a highly diverse bulk inoculum from the 2006 and 2007 St. Paul buckthorn nursery, 49 accessions (~14%) were resistant. Many of these accessions were heterogeneous in reaction, but several accessions were uniformly highly resistant in all tests. Resistant accessions were found from throughout much of the natural range of *A. barbata*. Initial crosses of some of the better accessions have been made to cultivated oat. We have also initiated screening of the Canadian germplasm collection of *A. barbata*, and similar levels of crown rust resistance are being observed.

Control of pineapple fusariosis with liquid tannins of *Acacia mearnsii*

R. CARVALHO (1)
(1) EMEPA (Empresa Estadual de Pesquisa Agropecuária da Paraíba) - João Pessoa - Paraiba - Brazil
Phytopathology 98:S31

Brazil is the largest pineapple (*Ananas comosus* var. *comosus*) producer in the world with high yields and excellent fruit quality. However, despite the development of technologies for this crop, huge losses still occur due to high incidences of fusariosis disease, a devastating fruit rot caused by the fungus *Fusarium subglutinans* f. sp. *ananas* which attacks not only the fruit, but also the whole plant and its slips. An alternative control program for pineapple fusariosis has been carried out since 1996 at the Pineapple Experimental Station at Sapé, Paraíba, Brazil, in which tannins of medicinal trees have been tested against the fungus *F. subglutinans* f. sp. *ananas* in laboratory and field experiments. However, despite the existence of many tree species rich in tannins in Brazilian rainforests, severe environmental regulations prohibit their utilization in agricultural IPM programs. Therefore, a new line of research had been carried out since 2001 with the use of tannins of cultivated black wattle trees (*Acacia mearnsii*) from which tannins are extracted, traded in a powdered form and used worldwide in the tannery and leather industries. Despite their efficiency, powdered tannins left residues around pineapple flowers giving the fruit an old appearance, unsuitable for commercialization. Thus, with the release of new commercial tannin products in liquid form by the tannin extraction industries, another field experiment was carried out in 2007 in a randomized block design with four replicates, to test their efficiency in reducing pineapple fusariosis. Evaluations at harvest showed a significant reduction in the incidence of pineapple fusariosis from 21.0% in the control treatment to 7.1% in the liquid tannin treatment, without leaving pigmentation or residues on the fruit. Besides being environment friendly, this tannin approach to control pineapple diseases could also be extended to other *Fusarium* diseases, being an invaluable tool to a modern, integrated Phytopathology.

Control of black rot of pineapples with calcium oxide

R. CARVALHO (1)
(1) EMEPA (Empresa Estadual de Pesquisa Agropecuária da Paraíba) - João Pessoa - Paraiba - Brazil
Phytopathology 98:S31

The black rot of pineapple (*Ananas comosus* var. *comosus*) is an important post-harvest disease which is common in almost all producing areas of the world. It is caused by the fungus *Chalara* (*Thielaviopsis*) *paradoxa* which penetrates mainly through wounds made on the fruit base during harvest. Infection is rapidly followed by large dark lesions and by a huge rot which characterize this disease. Different from other pineapple pests and diseases which are treated with pesticides some months before the harvest, the control of black rot of pineapple requires the application of fungicides immediately after the harvesting process, leaving a short time between spraying and consumption with high risks of contamination. This work had the objective of studying the effect of alternative products on the control of this disease, including the use of liquid wax, tannins, citrus extracts, food preservatives (used in bakery and fruit juice industries) and calcium oxide (lime). This research was carried out at the Pineapple Research Station in Sapé, Paraíba, Brazil, in 2007, in a completely randomized experimental design. Most of the alternative products were incapable of preventing the development of the disease. However, it was found out that the use of calcium oxide (lime)

applied as a paste as well as in a powdered form did not allow the establishment of the disease nor the development of lesions. Therefore, this research has shown that an environment friendly alternative control of black rot of pineapple can be achieved by using only calcium oxide (lime). Furthermore, since each pineapple fruit must be individually sprayed with chemical fungicides immediately after harvesting in order to prevent infection through the wounds, this lime approach will avoid the risk of contamination to consumers, besides being an old technique turned into a rediscovered contribution to a modern integrated Phytopathology.

Molecular characterization of virulence and pathogenicity determinants in *Xanthomonas axonopodis* pv. *manihotis*
L. F. CASTIBLANCO (3), C. A. Trujillo (3), N. C. Alfonso (3), C. Lopez (2), B. Szurek (1), V. Verdier (1), S. Restrepo (3), A. J. Bernal (3)
(1) IRD, Montpellier, France; (2) Universidad Nacional, Bogota, Colombia; (3) Universidad de Los Andes, Bogota, Colombia
Phytopathology 98:S32

Cassava Bacterial Blight (CBB) is the most important bacterial disease affecting cassava growing areas around the world. Knowledge about the molecular strategies used by *Xam* to cause disease is limited. Determinants of pathogenicity in other *Xanthomonas* have been identified. These include the production of Exopolysaccharides (EPS) and cell wall degrading enzymes (CWDE), as well as the injection of effector proteins into the host cytoplasm. The expression of some of these factors during infection is controlled via Quorum sensing mechanisms. Only one type III effector gene, *pth*B, has been reported in this bacterium and its presence has been correlated with pathogenicity. This gene encodes a protein belonging to the AvrBs3 family. We are using a mutagenesis approach to determine the importance of this and other potential pathogenicity genes in disease development. We generated a *pth*B mutant in the CFPB1851 strain of *Xam* using mutagenesis by double crossing-over. Additionally, we constructed different versions of the gene which lack different domains in the protein in order to initiate structure-function studies on the PthB protein. Mutants for other pathogenicity determinants, including genes controlling Quorum sensing, EPS and CWDE production have been generated using single crossing over. The pathogenicity of these mutants was assessed by inoculation in greenhouse and *in vitro*-grown plants. This work is an approach to characterize the virulence and pathogenicity factors in this bacterium.

Assessing the detection efficiency of the different sources of primary inoculum of rice sheath blight (*Rhizoctonia solani* Kühn) in the soil at different flooding durations using mungbean seedling – based tests
N. P. CASTILLA (1), S. Savary (1)
(1) International Rice Research Institute
Phytopathology 98:S32

The efficiency of different and simple methods to assess the primary inoculum of rice sheath blight (*Rhizoctonia solani*) in the soil was studied and analyzed using logistic regressions and receiver operating characteristic (ROC) curve analyses. The fraction of diseased mungbean seedlings (FDS) and the fraction of diseased seedlings and ungerminated seeds (FDSU) were assessed as inoculum predictors in two trials. Areas under the ROC curve were 0.754 and 0.859 for FDS and FDSU, respectively, in the first trial, and 0.778 and 0.792 for FDS and FDSU, respectively, in the second trial. The efficiencies of the predictors in detecting inoculum were compared among the different sources of inoculum, in two different soils, and with three flooding durations (flash flooding, one week flooding and two weeks flooding). The sources of inoculum were sclerotia, rice stems, and rice grain/hull mixture (RGHM). Rice stems and RGHM represented mycelium in colonized crop residues. Controls were uninoculated soil, where sclerotia or mycelium may naturally occur. The efficiency of mungbean seedling predictors (diseased seedlings, ungerminated seeds) in detecting inoculum did not significantly differ between soils. It was, however, significantly affected by flooding duration, source of inoculum, and their interaction. The efficiency of detecting sclerotia was not affected by flooding of up to two weeks. After one week of flooding, the efficiency of detecting colonized stem did not change but that of RGHM decreased by 57%. After two weeks of flooding, the detection of inoculum consisting of colonized stems and RGHM declined by 52% and 89%, respectively. Sclerotia may be a more aggressive and resilient source of primary inoculum in irrigated rice fields than mycelium. However, the decline of inoculum detection with flooding in uninoculated soils suggests that the primary, naturally occurring, inoculum may consist of a mixture of sclerotia and mycelium in colonized crop residues.

Isolation and identification of fungi associated with reniform nematode *Rotylenchulus reniformis*
J. D. CASTILLO (1), K. Lawrence (1), G. Morgan Jones (1)
(1) Auburn University, Auburn, AL, USA
Phytopathology 98:S32

The objective of this research was to identify fungi colonizing *Rotylenchulus reniformis* vermiform life stages and eggs. Soil samples from aged greenhouse cotton *R. reniformis* stock plants and from cotton fields in three different counties in Alabama, (Escambia, Limestone, and Baldwin) were collected. Nematodes were extracted by gravity screening followed by sucrose centrifugation. Vermiform nematodes and eggs with dark coloration or mycelial colonization were aseptically cultured on 1.5% of water agar supplemented with chlorotetracycline and streptomycine sulfate. *Arthrobotrys dactyloides*, *Dactylaria brachophaga*, *Paecilomyces lilacinus*, and *Fusarium oxysporum* were isolated from *R. reniformis* life stages. These fungi have been previously reported as nematophagous to other nematode species. Arthrobotrys dactyloides was found in two of the three field locations and colonized 48% of the *R. reniformis* in the greenhouse cultures. *Paecilomyces lilacinus* was isolated from 12% of vermiform life stages and 50% of eggs in greenhouse cultures, but was not found in the field locations. Other fungi associated with R. reniformins nematodes were: *Penicillium waksmanii*, *Phoma exigua*, *Aspergillus glaucus group*, *Cladosporium cladiosporioides*, *Cladosporium herbarum*, *Torula herbarum*, and *Aspergillus fumigatus.*

A comparison of Standard and High-fidelity PCR in the detection of *Pseudocercospora odontoglossi* from *Cattleya* orchids
R. A. CATING (1), M. A. Hoy (1), A. J. Palmateer (1)
(1) University of Florida
Phytopathology 98:S32

The polymerase chain reaction (PCR) has been used with increased frequency for detecting and identifying plant pathogens. Although the PCR is sensitive, research has shown that amplification of target microbial DNA from within another organism, such as an arthropod or plant, can be inhibited by the presence of host DNA. High-fidelity PCR (=Long PCR), which incorporates a second DNA polymerase with proofreading ability, has been shown to be more sensitive than Standard PCR in detecting *Wolbachia* DNA from within its arthropod host and in detecting greening bacteria from within citrus leaves and psyllids. This study compared the sensitivity of Standard PCR and High-fidelity PCR in detecting and amplifying DNA from the fungal pathogen *Pseudocercospora odontoglossi* in the presence of *Cattleya* orchid DNA. Different dilutions of plasmids containing the ITS1, 5.8s rDNA, and ITS2 from *P. odontoglossi* were spiked with *Cattleya* orchid plant DNA. High-fidelity PCR could detect and amplify as few as 207 plasmids containing the fungal DNA, while Standard PCR required over 2 million. Currently, we are comparing the ability of Standard and High-fidelity PCR to amplify fungal DNA taken directly from symptomatic plants. The use of High-fidelity PCR for the detection of pathogens *in vivo* could greatly improve our ability to identify pathogens that have been difficult to detect, as well as pathogens of regulatory significance.

Cultural techniques for growth and sporulation of *Pseudocercospora dendrobii* isolated from *Dendrobium* orchids
R. A. CATING (1), C. M. Stiles (1), A. J. Palmateer (1)
(1) University of Florida
Phytopathology 98:S32

Some cercosporoid fungi are notorious for slow growth and reduced conidial production when grown on artificial media. While some species may grow and sporulate readily in culture, others require special media, light, or temperature for growth and conidia production. In this study, single spore isolates of *Pseudocercospora dendrobii* taken directly from diseased orchids were grown on 9 different media: *Dendrobium* orchid leaf agar (DOLA) without added amino acid, DOLA + leucine, DOLA + asparagine, DOLA + alanine, quarter strength V-8, full strength V-8, PDA, water agar, and water agar containing autoclaved *Dendrobium* orchid leaf tissue. Plates were incubated at 25°C or 30°C under 12 h light, 12 h dark. Mycelial growth was measured weekly for 3 weeks, and plates were examined for sporulation weekly for 8 weeks. Mycelial growth was greatest on V-8 at 25°C, while sporulation occurred only on water agar containing autoclaved *Dendrobium* orchid leaf tissue at 25°C. No growth was observed on any media at 30°C. These results have allowed us to produce spores in axenic culture to be used in pathogenicity and host range studies, morphological studies, as well as produce mycelia on V-8 juice agar for use in phylogenetic analysis.

Occurrence of *Dickeya chrysanthemi* (*Erwinia chrysanthemi*) on *Vanda* orchids in Florida
R. A. CATING (1), J. C. Hong (1), A. J. Palmateer (1), C. M. Stiles (1), E. R. Dickstein (1)
(1) University of Florida
Phytopathology 98:S32

Vanda orchids are epiphytes cultivated for their attractive flowers by commercial producers and hobbyists throughout Florida. In August 2007, five *Vanda* hybrids were found at a nursery in central Florida with leaves that were macerated, brown, and water soaked. Isolated bacteria grew at 37°C, were

gram negative, and tested positive for pectolytic activity and phosphatase. MIDI (Sherlock version TSBA 4.10; Microbial Identification (16 System, Newark, DE) (SIM 0.906) identified the bacteria as *Erwinia chrysanthemi* (*Dickeya chrysanthemi* Burkholder et al. 1953) Samson et al. 2005. A PCR was performed on the 16S rRNA gene. Subsequent DNA sequencing and GenBank search showed the isolated strain is 99% identical to that of *Dickeya chrysanthemi*. Pathogenicity was confirmed by injecting approximately 150 µl of a bacterial suspension at 1×10^8 CFU/ml into 4 leaves each of six *Vanda* hybrids. One plant was inoculated with water in each of 4 leaves. Plants were enclosed in plastic bags and returned to the greenhouse. Within 24 h soft-rot symptoms appeared on inoculated leaves. The water control appeared normal. *Dickeya chrysanthemi* was re-isolated and identified using the above method, fulfilling Koch's postulates. To our knowledge, this is the first report of a soft-rot caused by *Dickeya chrysanthemi* on *Vanda* hybrids. Research is currently being conducted to determine the ability of this isolate to infect other commonly cultivated members of the Orchidaceae.

Molecular evolutionary analysis of resistance gene eIF4E and creation of novel resistance alleles in potato

J. R. CAVATORTA (1), K. W. Perez (1), M. Jahn (2), S. Gray (1)
(1) Cornell University, Ithaca, NY, USA; (2) University of Wisconsin, Madison, WI, USA
Phytopathology 98:S33

Resistance to viruses has long been an important breeding objective for researchers working with a number of different crops. As molecular techniques have identified the genes underlying virus resistance it has become increasingly apparent that the eukaryotic translation Initiation Factor 4E (eIF4E), a protein involved in recruiting RNA to the ribosomal complex, is a common resistance mechanism in a diversity of plant taxa against a number of virus families. Potato Virus Y (PVY) is the most important viral disease of potato. Resistance at the eIF4E locus has provided resistance to PVY and other Potyviruses in a number of species including potato relatives such as pepper and tomato. The work of our lab involves the transgenic expression of these resistance genes from pepper to confer virus resistance in other species. My particular area of research has involved a molecular evolution study of the eIF4E gene. By analyzing differences in the rates of synonymous and nonsynonymous amino acid substitutions I have found that several sites of the eIF4E gene are predicted to have undergone strong positive selection and that these sites correspond significantly with codons known to be involved in virus resistance. In addition, I have generated site-directed mutagenesis of the susceptible potato gene to create a potato allele predicted to confer resistance to PVY. I have tested that these novel alleles disrupt the interaction between eIF4E and viral pathogenicity determinants and I am currently expressing these modified potato alleles in potatoes to try and engineer resistance using DNA sequences obtained from within the species. The end goal of this study is to develop a virus resistance potato cultivar with improved consumer acceptance over previous cultivars that have utilized pathogen-derived resistance.

Unraveling the mechanism of ascospore discharge in *Fusarium graminearum*

B. Cavinder (1), H. Hallen (1), F. TRAIL (1)
(1) Michigan State University, East Lansing, MI, USA
Phytopathology 98:S33

Forcible ascospore discahrge is an important dispersal mechanism among the pathogenic fungi, yet the mechanism of ascus function is largely unexplored. We have used physiological, genetic and genomic approaches to understand ascus structure and function in the wheat pathogen, *Fusarium graminearum.* The turgor pressure that drives discharge relies on buildup of K^+ and Cl^- ions. We have identifed two genes that serve to regulate ascus firing, as determined by mutational analysis. Further investigations are aimed at the targets of this regulation. Expression analysis during perithecium development using Affymetrix GeneChips has identified genes expressed during ascus formation. Further analysis of these genes will reveal important components of ascus structure. Together, these data reveal a complex cellular structure that allows the ascus to fire its spores.

Characterization of mutant phenotype and downstream targets of *ust1*, an *Aspergillus* StuA like transcription factor in *Ustilago maydis*

N. CHACKO (1), S. Gold (1)
(1) University of Georgia, Athens, GA, USA
Phytopathology 98:S33

The fungus *Ustilago maydis* is the causal agent of corn smut. The pathogen is an important model for plant pathogenic basidiomycetes. The formation of filamentous dikaryon following mating is necessary for infecting plants. Further development and formation of dark diploid teliospores occurs only in the host. The deletion of *ust1*, the ortholog of an Aspergillus transcription regulator *stuA*, resulted in a filamentous mutant that surprisingly produces teliospore like structures in culture. This suggests that *ust1* is a master regulator in the dimorphic switch and sporulation pathway. The main aim of this project is to study the gene network involved in teliospore development using *ust1* as a surrogate system. The specific aims of this study are: 1. To determine the role of *ust1* in regulation of genes down-regulated in filamentous stage: Thirty-seven genes were identified as down-regulated in the filamentous cells in a previous study. Of these, 13 have putative binding sites for *ust1*. To date deletion constructs have been made for all these genes and three of the genes have been deleted using the DelsGate technique. 2. To determine the interaction of *ust1* with other components in the sporulation pathway using genetic suppressor analysis: Around 100 suppressor mutants have been created by UV irradiation. These mutants have been classified into phenotypic groups and are the focus of complementation experiments using a genomic DNA library. 3. To determine the effect of *ust1* mutation on global gene expression: Microarray analysis using *ust1* at two time points in comparison with wildtype has been initiated. Current results from the above aims will be presented.

Determination of the population structure of *Rhizoctonia oryzae-sativae* from paddy rice fields in California by microsatellite analysis

P. CHAIJUCKAM (1), C. A. Greer (2), R. Davis (1)
(1) Department of Plant Pathology, University of California, Davis, CA, USA; (2) UC Cooperative Extension, 142 Garden Highway, Suite A, Yuba City, CA, USA
Phytopathology 98:S33

The population structure of *Rhizoctonia oryzae-sativae*, the cause of aggregate sheath spot disease on rice, among three locations in California was determined in 2004 to 2006 by six single locus microsatellite markers. There was no significant population subdivision among sampling years based on analysis of molecular variance (AMOVA). However, there was small population structure among locations within sampling years, among fields within locations and within fields. These results indicated high gene flow among populations which was also supported by little to no differentiation (F_{ST}) and low genetic distance between pairs of locations. Furthermore, sexual reproduction was a major mode of reproduction in each location with respect to linkage equilibrium of most markers and a high degree of gene diversity and genotypic diversity. Asexual reproduction played a minor role in the short distance dispersal of the fungus within fields. Because a high level of gene flow and few asexual propagules were shared among populations, basidiospores were most likely the main cause of gene flow among populations, including short and long distances. This information may be important in the development of control strategies.

The role of Glycerol metabolism in the Arabidopsis-Colletotrichum higginsianum interaction

B. CHANDA (2), S. Venugopal (2), S. Kulshrestha (2), Q. Gao (2), D. Navarre (1), B. Downie (2), A. Kachroo (2), L. Vaillancourt (2), P. Kachroo (2)
(1) U.S. Department of Agriculture–Agricultural Research Service, Washington State University, Prosser, WA 99350; (2) University of Kentucky, Lexington, KY, USA
Phytopathology 98:S33

Previously, we have shown that a balance between glycerol-3-phosphate (G3P) and oleic acid levels is critical for defense signaling in Arabidopsis. We are now testing the hypothesis that changes in levels of glycerol, and its products, resulting from pathogen metabolic activity represents a novel signal triggering defense responses in Arabidopsis. Our approach has been to manipulate glycerol metabolic genes in Arabidopsis and in Colletotrichum higginsianum, cause of crucifer anthracnose. Inoculation with C. higginsianum increases host G3P and concomitantly declines glycerol levels, suggesting a role for these metabolites in the host-pathogen interaction. To further elucidate the role of glycerol-metabolism in host defense and pathogenesis, we evaluated the response of mutants affected in various steps of glycerol metabolism. In addition, a strain of C. higginsianum with a knock-out mutation in the G3P dehydrogenase gene (g3pdh) was produced. The g3pdh KO strain grew better when the medium was supplemented with glycerol, and the growth rate increased with increasing glycerol concentrations. The mutant strain accumulated reduced amounts of glycerol, and showed a reduction in appressorial turgor. Interestingly, while the g3pdh KO was less aggressive on wild type Arabidopsis, its infectivity was restored on various Arabidopsis mutants impaired in glycerol metabolism. Our results support a role for glycerol and G3P in the interaction between Arabidopsis and C. higginsianum.

Nutritional requirements of *Xylella fastidiosa* that causes bacterial leaf scorch of blueberry

C. CHANG (1), M. Ahmed (1)
(1) University of Georgia, Griffin, GA, USA
Phytopathology 98:S33

Two undefined media (PW and CS20) and one defined medium (XF-26) were compared for their abilities in supporting the primary isolations of *Xylella*

fastidiosa (Xf) from tissues of four diseased blueberry plants. One gram of stem tissues from each plant were sterilized with 15% Clorox for 3 min before being rinsed 3 times in sterile water. Each tissue was minced in 3 mL of PW broth; 0.1 mL of the minced sap was used for a 10-fold serial dilution in PW broth to 10^{-8}. One tenth of one mL of each cell supension from the following dilutions was placed onto the three agar media and spread with an L-shaped glass rod: 10^{-3}, 10^{-4}, and 10^{-5}. CFUs per plate per dilution were counted and were then converted to CFUs per gram of tissues. The average CFUs/g tissues were 8.6×10^6, 8.5×10^6, and 9.0×10^5 for PW, CS20, and XF-26 respectively. Of the 17 amino acids in XF-26 medium, 8 belong to nonpolar R groups (designated as E), 6 uncharged polar R groups (F) which were reportedly able to support the growth of Xf of Pierce's disease strains and 3 positively charged polar R groups (G). With various combinations of amino acid groups, 7 broth media were made for the growth of Xf; they were XF-26, XF-26E, XF-26F, XF-26G, XF-26EF, XF-26EG, and XF-26FG. Seven serial 10-fold sub culturing weekly in each medium were performed to determine their ability of supporting growth of Xf. XF-26, XF-26E, XF-26EF, and XF-26EG supported the growth of Xf whereas the other three did not which indicated the medium with 8 nonpolar R group amino acids was sufficient for the growth of blueberry Xf. XF-26E was hence shown to be able to support the primary isolation of Xf from diseased blueberry tissues.

Development of a molecular marker for specific detection of *Fusarium oxysporum* f. sp. *cubense*, a causing agent of banana wilts disease

P. CHANG (1), Y. Lin (1), J. Chang (1), E. Liu (1), C. Chao (2), J. Huang (1)
(1) Department of Plant Pathology, National Chung Hsing University, Taichung City, Taiwan 402, R. O. C.; (2) Division of Banana Cultivation and Post-harvest Physiology, Taiwan Banana Research Institute, Pingtung, Taiwan 904, R. O. C.
Phytopathology 98:S34

Fusarium wilt of banana (*Musa* spp.), commonly known as Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder & Hansen (Foc), is one of the most severe fungal diseases in banana, and reported to be one of the major limiting factors for banana production worldwide. Foc can survive in soil as a saprophyte for many years, and it is very difficult to eradicate this soil-borne disease. Thus, rapid and reliable diagnosis and pathogen detection is the foundation of Fusarium wilt control in banana production. In order to accelerate the detection process and to increase the sensitivity of detection strategy, we have developed a reliable PCR technique to detect Foc isolates in Taiwan. With optimized PCR assay, the molecular method was sensitive and could detect small quantities (10 pg) of Foc DNA in 50 to 2000 ng host genomic DNA without affecting the amplification efficiency. We also demonstrated that by using our PCR assay, Foc pathogen could be easily differentiated from other *formae speciales* of *F. oxysporum*. (supported in part by BAPHIQ, and Department of International Affairs, Council of Agriculture, Taiwan, R.O.C. under grant numbers 89ST-6.2-BQ-65(06), 91AS-7.3.1-BQ-B2(3), 93AS-1.9.2-BQ-B1, and 96AS-4.1.2-IC-I1(2); by the Ministry of Education, Taiwan, R.O.C. under the ATU plan; and by National Chung Hsing University, Taiwan, R.O.C.)

Characterizing soybean rust resistance in *Glycine tomentella*

S. CHANG (2), T. A. Steinlage (2), T. Hymowitz (2), G. L. Hartman (1)
(1) USDA-ARS, Urbana, IL, USA; (2) University of Illinois, Urbana-Champaign, IL, USA
Phytopathology 98:S34

Phakopsora pachyrhizi Syd., the causal agent of soybean rust, is a widespread and damaging pathogen found throughout tropical and sub-tropical regions of the world. The pathogen has a broad host range that includes the perennial *Glycine* species. Some accessions within these species are known to carry single dominant or multiple dominant single genes for resistance to *P. pachyrhizi*. Our objective was to screen *G. tomentella* accessions for rust resistance and to evalute the genetics of this resistance. Greenhouse grown *G. tomentella* plants were spray-inoculated with a soybean rust isolate. Eleven accessions were resistant and three accessions were susceptible, allowing the fungus to sporulate profusely. When screened by a detached leaf assay, four susceptible lines were found. Four resistant accessions and one susceptible accession were reciprocally crossed and F2 progeny were screened for resistance. Segregation analysis indicated that resistance was not the result of a single dominant gene. Thus, rust resistance in *G. tomentella* may represent multiple unique genes for resistance to *P. pachyrhizi* not found in soybean.

Competitive studies on parasitic fitness using blended soil infestations of mefenoxam-sensitive and mefenoxam-resistant *Phytophthora erythroseptica* isolates under fungicide selection pressure

V. CHAPARA (1), R. J. Taylor (1), J. S. Pasche (1), N. C. Gudmestad (1)
(1) Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58102
Phytopathology 98:S34

The oomycete *Phytophthora erythroseptica*, cause of potato pink rot, is known to have a wide range of sensitivities to the fungicide mefenoxam. An experiment was conducted in the field and under *in vivo* conditions in potato to study competitive ability (parasitic fitness) of one resistant (EC_{50} > 100 μ ml^{-1}) vs. one sensitive (EC_{50} = 0.07 μ ml^{-1}) isolate of *P. erythroseptica*, under the influence of mefenoxam and non-mefenoxam (phosphorous acid) fungicides. These two isolates were determined in preliminary studies to be equally aggressive in nontreated potato tubers. Isolates were blended at proportions of 1:1, 3:1, and 1:3 resistant:sensitive and introduced into the soil at planting. Non-inoculated, non-fungicide-treated plots served as controls. In furrow mefenoxam applications were applied to the soil immediately following infestation with *P. erythroseptica* as hilling disks were closing the furrow and phosphorous acid was applied to the foliage at tuber set and 14 days after tuber set. *P. erythroseptica* isolates were recovered from pink rot infected tubers at harvest and four weeks after harvest and were tested for mefenoxam sensitivity *in vitro*. For *in vivo* studies, single zoospores were challenge inoculated onto field harvested potato tubers of nontreated noninoculated, phosphorous-acid treated noninoculated and mefenoxam-treated noninoculated treatments. The competitive abilities of both sensitive and resistant isolates of *P. erythroseptica* were similar in the absence of fungicide pressure. With selection pressure from mefenoxam, resistant isolates were more competitive than sensitive isolates, as expected. In contrast, under selection pressure of a non-mefenoxam fungicide (phosphorous acid), mefenoxam sensitive isolates competed well with resistant isolates.

The role of anastomosis in the sexual development of *Epichloë* endophytes

N. D. CHARLTON (1), K. D. Craven (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK
Phytopathology 98:S34

Vegetative hyphal fusion (anastomosis) plays an important role in vegetative growth and sexual development in filamentous fungi. A *soft* (*so*) mutant of *Neurospora crassa* and *aso-1* mutant of *Alternaria brassicicola* have been shown to be incapable of self-fusion. The inability to create interconnected networks of self-anastomosed hyphae may prohibit a fungus from producing structures of complex organization, such as sexual fruiting bodies. *Epichloë* endophytes are intimate fungal symbionts of cool-season grasses that generally grow as sparse, unbranched hyphae in the intercellular spaces of host tissues. However, these endophytes have an obligate sexual stage which occurs concurrent with the onset of host inflorescence development, wherein the mycelium emerges as dense epiphytic growth that surrounds the host flower and causes its abortion. The synchronization of the sexual cycle of *Epichloë* endophytes with inflorescence development in the host may be associated with a switch from non-anastomosed to anastomosed tissue. In this study we are testing whether 1) the transition from the vegetative to the sexual stage of the *Epichloë* lifecycle is mediated by the *so* gene and 2) a signal of plant origin is involved in the initiation of this fungal lifestyle transition. We are using nitrate-nonutilizing (*nit*) mutants to study the effects of plant signals (e.g. simple sugars, gibberellins) on self-anastomosis, which have been shown to play a role in the control of flowering. Work is in progress to elucidate the role of *so* in *E. festucae* and *E. typhina* by assessing the ability of knockout mutants to self-anastomose and express the sexual stage *in planta*.

Using remote sensing to evaluate the efficacy of inoculative biocontrol

S. CHAUDHARY (1), A. Davelos Baines (1), K. Summy (1), C. R. Little (2)
(1) Department of Biology, The University of Texas – Pan American, Edinburg, TX; (2) Department of Plant Pathology, Kansas State University, Manhattan, KS
Phytopathology 98:S34

The efficacy of *Streptomyces* isolates for biological control of gummy stem blight (GSB) of cantaloupes (*Cucumis melo* var. 'Primo') was evaluated using remote sensing for both *in vitro* and greenhouse pot trials. Mortality of plants infected with the causative agent of GSB (*Didymella bryoniae*) was evaluated in the presence and absence of selected *Streptomyces* isolates. Comparison among treatments indicated a significant impact upon plant mortality for each of three *Streptomyces* isolates (MA1F4#2, WI1B#5, and MA2A4 #2), which appear to be most effective when used in combination. The near infrared to red (NIR/R) ratios of infected plants treated with *Streptomyces* were similar to uninfected controls providing evidence for effective biological control. Further, substantially higher NIR/R ratios were observed for infected plants growing in the presence of *Streptomyces* compared to infected plants growing in the absence of *Streptomyces* isolates. These results suggest potential efficacy of *Streptomyces* for biological control of *D. bryoniae* and demonstrate potential applications of remote sensing technology in evaluation of biocontrol agents for plant pathogens.

The expression of maize 14 kDa trypsin inhibitor protein on host resistance to *Aspergillus flavus* infection and aflatoxin production

Z. CHEN (1), R. Brown (2), T. Cleveland (2), K. Damann (1)
(1) Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803; (2) Southern Regional Research Center, USDA ARS, New Orleans, LA 70179
Phytopathology 98:S35

Maize (*Zea mays* L.) is one of the major crops that are susceptible to *Aspergillus flavus* infection and subsequent contamination with aflatoxins, the most potent carcinogenic secondary metabolites of the fungus. Maize genotypes resistant to *A. flavus* infection and/or aflatoxin contamination have been identified and compared with susceptible genotypes for differences in protein profiles. Several resistance-associated proteins have been found and sequenced. One of them is identified as a 14 kDa trypsin inhibitor (TI). To further investigate whether the high level expression of TI plays a direct role in host resistance of maize to *A. flavus* infection and/or aflatoxin production, an RNAi gene silencing vector was constructed using the Gateway technology and transformed into the immature maize embryos in the present study. Sixty-six transgenic ears representing 18 independent transformation events were produced. Twenty ears representing 10 independent transformation events were selected for further evaluation. Kernels from 12 ears were confirmed positive for transformation. The TI production was also reduced by 10 to over 85% in the RNAi silenced transgenic kernels compared to control lines when analyzed using proteomics. Kernel screening assay also revealed that the transgenic kernels with reduced levels of TI production were more susceptible to *A. flavus* colonization and aflatoxin production. This study demonstrated the importance of the 14 kDa trypsin inhibitor expression in maize resistance to *A. flavus* infection and/or aflatoxin production.

Detection of phytoplasma and Candidatus Liberibacter asiaticus in citrus showing Huanglongbing (yellow shoot disease) symptoms in Guangdong, P. R. China

J. CHEN (2), X. Deng (1), S. Liu (1), X. Pu (1), H. Li (1), E. Civerolo (2)
(1) South China Agricultural University, Guangzhou, China; (2) USDA-ARS, Parlier, CA, USA
Phytopathology 98:S35

Huanglongbing (HLB) or yellow shoot disease (ex. greening disease) is highly destructive to citrus production worldwide. HLB is currently known to be associated with Candidatus Liberibacter asiaticus in China. However, Koch's postulates have not been fulfilled. It also remains unclear if other plant pathogens are involved. In two surveys performed in Guangdong, P. R. China in 2006 and 2007, 141 samples were collected from citrus trees showing typical symptoms of HLB from 11 different cities. PCR using phytoplasma specific primer sets fU5/rU3 nested with primer set P1/P7 identified 110 (78.0%) positive samples. A 1,785 bp amplicon was obtained with primer set P1/P7 and showed a 100% identity to three strains of *Ca.* Phytoplasma asteri (onion yellows (Japan), aster yellows 'watercress' (Hawaii), and valeriana yellows (Lithuania). Meanwhile, 89 (63.1%) samples were positive for *Ca.* Liberibacter asiaticus. Sixty-nine (48.9%) samples were positive to both phytoplasma and Ca. L. asiaticus. Transmission electron microscopy (TEM) showed both walled and wall-less bodies in symptomatic citrus tissue. HLB phytoplasma was further demonstrated using periwinkle (*Catharanthus roseus* (L.) G. Don.) through dodder (*Cuscuta campestris* Yunck) transmission. PCR detected the same phytoplasma in the affected periwinkle, along with *Ca.* L. asiaticus. In addition to yellowing/mottling, the infected periwinkle showed typical symptoms of virescence and phyllody that are commonly associated with phytoplasmal diseases. TEM revealed bacteria-like organisms with pleomorphic morphology. Data from this study showed that in addition to *Ca.* L. asiaticus, a phytoplasma related to *Ca.* P. asteri was also associated with citrus HLB in Guangdong.

Variations of whole genome sequences of *Xylella fastidiosa* strains within the same pathotype

J. CHEN (2), G. Xie (1), S. Han (1), E. Civerolo (2)
(1) Los Alamos National Laboratory, Los Alamos, NM; (2) USDA-ARS, Parlier, CA, USA
Phytopathology 98:S35

Xylella fastidiosa is a Gram negative and nutritionally fastidious plant pathogenic bacterium that causes almond leaf scorch disease (ALSD) and Pierce's disease (PD) of grapevine. *X. fastidiosa* strains from almond can be divided into two pathotypes: ALSD-PD, represented by strain M23, and ALSD-only, represented by strains M12 and Dixon. Strains from grape belong to the ALSD-PD pathotype and represented by strain Temecula-1. Limited information is available regarding the genetic variations withing the same pathotype strains. Whole genome sequence comparisons between strains M23 and Temecula-1 and between strains M12 and Dixon showed high level of genomic similarities but gene variations were noticeable. We defined that a gene was considered to be strain-specific or unique if it had no hits with an E-value = 10^{-5} or less. With the ALSD-PD pathotype, strain M23 had 262 unique genes and strain Temecula had 30 unique genes. In contrast, 737 genes are identical with E-value = 0. Although the genome sequence of strain Dixon has not been closed, comparisons between strains M12 and Dixon showed that strain M12 had 105 unique genes and strain Dixon had 203 unique genes. Similarly, 713 genes were identical with E-value = 0. Among the unique genes, many of them were annotated as genes related to horizontal gene transfer. The genetic variations identified by whole genome sequence comparisons will have impact on future studies of strain differentiation, mechanisms of host specialization and genome evolution of *X. fastidiosa*.

Establishing model of nematodes as bioindicators for river pollution

W. CHEN (1), P. Chen (1), T. Tsay (1)
(1) Dept. Plant Pathology, National Chung-Hsing University, Taichung, Taiwan
Phytopathology 98:S35

Nematodes are ubiquitous and certain species are the most persistant in polluted or disturbed river environment. Correct methods of monitoring for river pollution are needed because of the tight relation between human life and river environment. The purpose of this study is to establish a model using nematodes as bioindicators for river pollution. Nematode communities of Beigang river in Taiwan catchments were investigated. A total 6995 nematodes were identified belonging to 18 families, 30 genera, 5 different c-p groups and 7 different feeding types. The two genera *Aphelenchoides* and *Eumonhystera* had the highest discriminatory power, showing a wide range of relative abundance in both water and sediments. The expected low Maturity Index values in the heavily polluted sediments with high River Pollution Index values were observed in this study. In upper and lower serious pollutant sites, Mohysteridae and Xyalidae are dominant population. Families and genera of nematodes were significantly different between sampling sites. Statistic analysis showed the kinds, abundances, MI values and feeding types of nematodes also had significant correlation with environment fators. Nematodes diversity was higher in downriver as well as during summer season, but there were no statistical differences between seasons. Our data suggested that BOD5, soil texture and pH were the mainly environment parameters to affect nematode communities. Nematodes were found in both clear and pollutant environments, in every soil type, under all climatic conditions, their communities had significant correlation with environment fators. Identification nematodes to the family level could be achieved by simple morphological characters. The sampling and extraction methods were relatively easy, with these advantages, this model could become a useful tool for monitoring river environments, habitats recovery and environmental biology studies.

Genetic diversity of *Sclerotinia sclerotiorum* from various crops from the U.S. Pacific Northwest

W. CHEN (2), L. Porter (1), D. Johnson (3)
(1) USDA ARS, Prosser, WA, USA; (2) USDA ARS, Washington State University; (3) Washington State University
Phytopathology 98:S35

Sclerotinia sclerotiorum causes white mold on many crops resulting in significant economical losses. Despite extensive studies on population variation of this pathogen in many crops, the populations of *S. sclerotiorum* in the U.S. Pacific Northwest (PNW) have not been extensively studied. The PNW harbors diverse cropping systems including irrigated and dry land agriculture on various geographical terrains with generally mild winter conditions. The different agricultural practices and cropping systems may impact population structure of *S. sclerotiorum*. This study was to examine genetic variation and population structure of *S. sclerotiorum* from different cropping systems in the PNW. Mycelial compatibility grouping was used to measure genetic diversity of 88 sclerotial isolates of *S. sclerotiorum* from three states (22 isolates from a potato field in Bonners Ferry, ID; 32 isolates from a potato field in Hermiston, OR; and 34 isolates from a pea field in Walla Walla, WA), to compare with isolates previously obtained from lentil. Each isolate was obtained at least 1.8 m away from other isolates collected within a field. All isolates from the same field were paired in all possible combinations. High levels of MCG diversity were found among the populations: 12 MCGs were found among 22 isolates from Bonners Ferry, ID, 20 MCGs among 32 isolates from Hermiston, OR, and 23 MCGs among 34 isolates from Walla Walla, WA. Relationship of genetic variation in neutral marker loci and variation in the quantitative phenotypic traits, pathogenicity and fungicide sensitivity, of the same populations is being investigated.

Characterization of seed-colonizing bacterial communities associated with the suppression of Pythium damping-off in a municipal biosolids compost

M. Chen (1), C. McGuire (1), E. B. NELSON (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA
Phytopathology 98:S36

We demonstrated previously that compost microbes colonizing seeds within 8 hr of sowing could explain Pythium suppressiveness in a municipal biosolids compost. In this study, we characterized seed-colonizing microbes to understand which taxa contribute directly to Pythium damping-off suppression. Selective inhibitors were applied to seeds to inactivate portions of the colonizing microbial community. After initial screenings for toxicity to both cucumber and *P. ultimum*, six selective inhibitors were chosen to assess their impacts on microbial populations and disease suppression. Rifampicin had little effect on fungal populations and was the most effective inhibitor for inactivating disease suppression. Bacterial communities from cucumber seeds sown in compost for 8 hr alone and either left non-treated, treated with 500 ppm rifampicin for 3 h, or treated with water for 3 h (control) were characterized using terminal restriction fragment length polymorphism (T-RFLP) analysis and compared. T-RFLP profiles revealed a wide diversity of distinct bacterial taxa on seed surfaces within 8 hr of sowing. Community profiles of non-treated and water-treated seeds were quite similar whereas community profiles from rifampicin-treated seeds differed. A limited number of terminal restriction fragments (TRFs) found on non-treated or water-treated seeds were absent in communities from rifampicin-treated seeds. Other TRFs differed quantitatively among treatments. Taxon assignments based on sequencing revealed the presence of a number of prokaryotes within the Actinobacteria, Firmicutes, and Bacteroidetes, some known to be suppressive to *Pythium* species.

Carbon competition as a mechanism of Pythium damping-off suppression in a municipal biosolids compost

M. Chen (1), E. B. NELSON (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA
Phytopathology 98:S36

The present study was initiated to determine whether fatty acid competition could explain how seed-colonizing microbial communities from a municipal biosolids compost suppress Pythium damping-off. A number of *P. ultimum* sporangium responses to seeds (i.e., sporangium germination, sporangial emptying, and germ tube abortion) were measured to determine the direct impacts of disease suppressive seed-colonizing microbial communities on *P. ultimum* behavior. Seed-colonizing microbial communities utilized cucumber seed exudate and linoleic acid *in vitro* to reduce *P. ultimum* sporangium germination. When sporangia were observed directly in the spermosphere, however, levels of sporangium germination and sporangial emptying in both the compost medium and sand did not differ. However, the percentage of aborted germ tubes was greater ($P < 0.005$) after a 12-h incubation in compost medium than the level of abortion among sporangia incubated in sand. Germinated sporangia supplemented with cucumber seed exudate did not form aborted germ tubes, whereas removal of cucumber seed exudate after various stages of sporangium germination resulted in an increase in aborted germ tubes over time. Addition of increasing levels of glucose directly to the compost medium suppressed germ tube abortion in the spermosphere and eliminated damping-off suppression. These data fail to support the role for linoleic acid competition in Pythium damping-off suppression but provide evidence for general carbon competition mediated by seed-colonizing microbial communities as a mechanism for the suppression of Pythium damping-off in a municipal biosolids compost.

Characterization of two peanut oxalate oxidase genes and development of peanut cultivars resistant to stem rot (*Sclerotium rolfsii*)

X. CHEN (3), T. Brenneman (3), A. Culbreath (3), C. Holbrook (1), B. Guo (2)
(1) USDA-ARS, Crop Genetics and Breeding Unit, Tifton, GA; (2) USDA-ARS, Crop Protection and Management Unit, Tifton, GA; (3) University of Georgia, Department of Plant Pathology, Tifton, GA
Phytopathology 98:S36

In the southeastern U.S., stem rot (*Sclerotium rolfsii*) is a common and destructive disease of peanut. Research has suggested the enhancement of resistance to Sclerotinia minor in peanut by expressing a barley oxalate oxidase gene. Oxalate oxidase belongs to the germin family of proteins and acts as a source of hydrogen peroxide (H_2O_2) in certain plant-pathogen interactions. We have identified and cloned two peanut endogenous oxalate oxidase genes, *AhOxOl*, originating from peanut leaf libraries, and *AhOxOs* from seed libraries. The goal is to characterize these two genes in resistance to *S. rolfsii*. The *AhOxOl* including 991 bp cDNA sequence encodes a 219 amino acid protein with a 21-residue signal peptide. After cleavage of the signal peptide, it has a mass of 20.84 kDa. The *AhOxOs* comprised of 744 bp cDNA encodes a protein with 220 amino acid residues containing a putative signal peptide of 24 residues, with a mass of 20.63 kDa after removal of the signal peptide. The two proteins both contain three motifs, Q/NDL/FCVAD, G(X)5HXH(X) 11G and G(X)5P(X) 4H(X) 3N, which are characteristic to germin-like proteins. Furthermore, the deduced protein of *AhOxOl* consists of the "germin box" (HI/THPRATEI), which is a conserved sequence shared by germins within the motif G(X)5HXH(X) 11G. Searches of GenBank database indicate that AhOxOl and AhOxOs, with approximately 37% of amino acid similarity to each other, exhibit respectively up to 76% and 82% amino acid identity to certain plant germin-like proteins. Southern blot analysis showed that the two genes possibly exist in at least four copies in the peanut genome. Northern blots conducted with total RNA from seed and leaf tissues of resistant and susceptible genotypes indicated that *AhOxOs* is mainly expressed in peanut seed. Further functional characterization will be conducted.

Identification and cloning of TSWV resistance gene(s) in cultivated peanuts and development of markers for breeding selection

X. CHEN (3), A. Culbreath (3), T. Brenneman (3), C. Holbrook (1), B. Guo (2)
(1) USDA-ARS, Crop Genetics and Breeding Unit, Tifton, GA; (2) USDA-ARS, Crop Protection and Management Unit, Tifton, GA; (3) University of Georgia, Department of Plant Pathology, Tifton, GA
Phytopathology 98:S36

Tomato spotted wilt virus, transmitted to plant via thrips, is a destructive pathogen with a worldwide distribution. TSWV has caused a very serious problem in peanut (*Arachis hypogaea* L.) producing areas in U.S. In past decades, different tactics (resistant cultivars, chemical, crop rotation and other field practices) have been employed to control spotted wilt. The most promising solution for managing spotted wilt is development of resistant cultivars. Resistance genes to TSWV have been found in tomato and pepper, named *Sw-5* and *Tsw*, respectively. We have discovered 41 gene fragments originally from peanut expressed sequence tags (ESTs) with significant homology to two tomato BAC sequences (AY007366 and AY007367), which spanned 5 different resistance candidate sequences. Reverse northern-blots have identified eighteen clones with significant levels of expression. Out of these clones, we identified one, named *Ahsw*, with approximately 37% of amino acid identity to tomato *Sw-a* that has been further characterized. Southern blot indicated that there are at least 4 copies of *Ahsw* gene in the cultivated peanut genome. Three restriction enzymes, *Hind*III, *Eco*RI and *Rsa*I were used to examine whether the restriction fragment length polymorphism exists in these entries, which are resistant or susceptible to TSWV, using *Ahsw* as a probe. The results showed that different number and length of restriction fragments were observed in different genotypes, suggesting its potential use as a marker. Two mapping populations have been developed. Northern blots revealed different expression patterns of *Ahsw*, but further experiments are needed to confirm an association of this gene with resistance to TSWV.

Virulent races of *Puccinia striiformis* identified in the United States in 2007

X. CHEN (2), A. Wan (1), K. Richardson (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, USA; (2) USDA-ARS and Department of Plant Pathology, Washington State University, Pullman, WA, USA
Phytopathology 98:S36

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (PST), and stripe rust of barley, caused by *P. striiformis* f. sp. *hordei* (PSH), were monitored by collaborators throughout the U.S. In 2007, stripe rust of wheat occurred in more than 16 states while stripe rust of barley was found in California, Colorado, and Washington. Both stripe rusts were generally in low levels except epidemic occurred in some areas. Urediniospores from infected leaf samples were increased on susceptible seedlings of either 'Nugaines' wheat or 'Steptoe' barley. Isolates of PST were tested on a set of 20 wheat differential genotypes and PSH isolates were tested on a set of 12 barley differential genotypes for identifying virulent races. Five PSH races and 30 PST races were detected. The most frequent races were PST-114 (virulent on Lemhi, Heines VII, Moro, Produra, Yamhill, Stephens, Lee, Fielder, Tres, Express, *Yr8*, *Yr9*, Clement, and Compair), PST-116 (all PST-114 virulences plus Paha), and PST-115 (all PST-116 virulence except for Moro). Among the 30 PST races, 11 were first identified in 2007. New race PST-127, which is the most virulent race identified to date, virulent on all 20 wheat differential genotypes except for Moro (*Yr10*, *YrMor*), *Yr5*, and Tres (*YrTr1*, *YrTr2*). This race has the combination of virulence on *Yr1*, *Yr2*, *Yr3a*, *Yr4a*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr19*, *Yr20*, *Yr21*, *Yr22*, *Yr23*, *YrExp1*, *YrExp2*, and several other resistance genes.

Pseudomonas syringae is equipped with diverse mechanisms to exploit choline and its analogs from plants

C. CHEN (2), A. A. Malek (1), M. J. Wargo (1), D. A. Hogan (1), G. A. Beattie (2)
(1) Dartmouth Medical School; (2) Iowa State University
Phytopathology 98:S37

As a pathogen and epiphyte of plants, *Pseudomonas syringae* has likely evolved mechanisms to exploit plant-derived choline and its analogs as carbon and energy sources and as osmoprotectants to help tolerate stress during plant colonization. In addition to the two osmoregulatory transporters BetT and OpuC, which transport choline and other substrates, *P. syringae* pv. tomato DC3000 has a catabolism-associated transporter for choline, which is encoded by PSPTO_0462-64, and we have designated it Bcc. The Bcc transporter is involved in the uptake of glycine betaine, choline, acetylcholine and probably carnitine, and is induced by the presence of choline and its analogs but not by hyperosmotic stress. Expression of the bcc genes requires the transcriptional regulator GbdR. Interestingly, in competitive inhibition assays, the uptake of radiolabeled substrates was not inhibited by distinct substrates, even when they were added at 100-fold higher concentrations than the labeled substrate, suggesting that this transporter has novel properties among the prokaryotic ABC transporters. Our continuing studies are exploring the ecological roles of these choline transporters during plant colonization by *P. syringae*.

Characterization of the *RB*-mediated late blight resistance phenotype

Y. CHEN (1), D. Halterman (2)
(1) University of Wisconsin-Madison; (2) University of Wisconsin-Madison, USDA-ARS
Phytopathology 98:S37

Late blight, caused by the oomycete pathogen *Phytophthora infestans*, continues to be the most devastating disease for potato cultivation. The resistance (*R*) gene *RB*, cloned from the wild potato species *Solanum bulbocastanum*, offers durable, broad spectrum and non-race specific resistance to late blight. To better understand the resistance mechanism mediated by *RB*, we examined the interaction between *P. infes*tans and transgenic *RB*-containing potato SP951 (*S. tuberosum* cv. *Katahdin* plus *RB*), using the susceptible potato *Katahdin* as the control. Three week old SP951 and *Katahdin* plants were inoculated with a high concentration (150,000 sporangia/ml) of *P. infestans* sporangia. Hypersensitive cell death (HR) was monitored at 0, 8, 12, 24, 48, and 72 hours after inoculation. Both micro-HR (only one or several neighboring cells) and macroscopic lesions were evident at each time point in the transgenic plants. In some cases, hyphae could be seen growing beyond the HR, demonstrating the partial resistance phenotype of this *R* gene. Leaves were harvested at the same time points for RNA extraction. Quantitative real-time reverse transcription PCR results showed that the transcriptional level of three pathogenesis related genes, *PR1 basic*, *PR2* and *PR5*, increased 8 hours after inoculation and then remained at this level until 72 hours after inoculation in both resistant SP951 and susceptible *Katahdin* plants. At each time point after inoculation, transcriptional levels of the three *PR* genes in SP951 and *Katahdin* were not significantly different. Our results suggest that *RB* is sufficient to induce HR immediately after inoculation, but lacks the ability to stop pathogen growth completely. Race non-specific *R* genes may use molecular mechanisms similar to the better-studied race specific *R* genes, but also have their unique way of mediating resistance.

***In vitro* sensitivity of *Waitea circinata* var. *circinata* to fludioxonil and polyoxin-D**

C. CHEN (1), F. P. Wong (1)
(1) University of California, Riverside, CA, USA
Phytopathology 98:S37

Waitea circinata var. *circinata* (Wcc) is the causal agent of brown ring patch, a *Rhizoctonia* disease of cool season turf species only recently documented in the U.S. Chemical control of the pathogen has been erratic in many locations and sensitivity of this pathogen to different classes of fungicides has only been partially characterized. Fludioxonil and polyoxin-D are phenylpyrrole and polyoxin fungicides, respectively, that can be effective for brown ring patch management. The objective of this study was to document the sensitivity of Wcc to these fungicides to better understand why these are effective against the pathogen in the field and to provide a sensitivity distribution that may be useful for future resistance monitoring. Twenty-five isolates of the pathogen were collected from diverse locations in the U.S. and tested using one-quarter strength potato dextrose agar amended with 0, 0.001, 0.01, 0.1, 1 and 10 mg/liter of fludioxonil or polyoxin-D. Petri plates of the amended agar were inoculuated with a 9-mm plug of Wcc (grown on ¼-PDA for 7 days) and incubated at 28°C for 3 days. Radial expansion was measured and used to calculate 50% effective dose (ED_{50}) values. ED_{50} values for fludioxonil ranged from 0.0067 to 0.74 mg/liter (mean = 0.15). ED_{50} values for polyoxin-D ranged from 0.054 to 0.75 mg/liter (mean = 0.23). No isolates were found to have qualitative resistance to either fungicide, and these distributions can serve as guides for determining possible shifts in fungicide sensitivity as a result of chemical management programs.

Characterization of growth and virulence-related genes expression of *Xylella fastidiosa* affected by grape xylem sap and cell-wall constituents

D. W. CHENG (1), H. Lin (1), A. M. Walker (2), D. C. Stenger (1), E. L. Civerolo (1)
(1) USDA, ARS, SJVARC, Parlier, CA, USA; (2) University of California, Davis, CA, USA
Phytopathology 98:S37

Pierce's disease (PD) of grapevines is caused by the xylem-limited bacterium *Xylella fastidiosa* (*Xf*). Most *vinifera*-based cultivars are susceptible to PD. However, grape species such as *V. arizonica*, *V. shuttleworthii*, *V. simpsonii*, *V. smalliana*, and *Muscadinia rotundifolia* are resistant to PD. In this study, we investigated effects of xylem sap collected from PD-resistant and PD-susceptible grape and several cell-wall constituents on bacterial growth, biofilm formation, and virulence-related gene expression *in vitro*. The results showed that xylem sap from PD-susceptible sources provided better support for bacterial growth and biofilm formation *in vitro* than sap from PD-resistant *Vitis* species. In bioassays of *Xf* bacteria, co-cultured with various purified cell-wall constituents, cellulose, xylan, laminarin, and glucan significantly promoted bacterial growth whereas lichenan strongly suppressed growth. However, only xylan, laminarin and k-carrageenan significantly promoted bacterial biofilm formation *in vitro*. Analyses of grape xylem sap protein and pathogenicity- and virulence-related gene expression showed that induced expression patterns were genotype- and treatment-specific, suggesting molecular interactions between xylem-limited bacteria and grape. This study demonstrates that differences in xylem cell wall properties and sap chemical composition may play important roles in PD development.

Transcriptional regulation of grape cytochrome P450 gene expression in response to *Xylella fastidiosa*

D. W. CHENG (2), H. Lin (2), A. M. Walker (1), D. C. Stenger (2), E. L. Civerolo (2)
(1) Department of Viticulture and Enology, University of California, Davis, CA 93616; (2) USDA-ARS-SJVARC, Parlier, CA 93648
Phytopathology 98:S37

Plant cytochrome P450 monooxygenases are versatile redox proteins that mediate biosynthesis of lignins, terpenes, alkaloids, and a variety of other secondary compounds as plant defense agents against a range of pathogens and insects. To determine if cytochrome P450 monooxygenases are involved in the defense response against infection by *Xylella fastidiosa* (Xf) and its regulatory mechanisms in grapevines, we investigated transcriptional and posttranscriptional modification patterns of a cytochrome P450 gene CYP736B in leaf and stem tissues of PD-resistant and susceptible grape plants after inoculation with this bacterium. Based on genomic DNA and cDNA cloning, the CYP736B gene is composed of two exons and one intron with GT as a donor site and AG as an acceptor site. Gene expression was up-regulated in PD resistant plants and down-regulated in PD-susceptible plants at 6 weeks postinoculation. 5′RACE and 3′ RACE analyses showed that there were 4 major upstream transcriptional regulatory (UTR) regions and 3 major downstream transcription regulatory (DTR) regions. The expression of cytochrome P450 CYP736B gene is regulated in response to Xf infection at both transcription and post-transcription levels. Selective usage of transcriptional initiation sites plays an important role in PD resistant grapevines against Xf infection by affecting transcriptional efficiency, pre-mRNA splicing, and mRNA maturation.

Sugarcane mosaic virus HC-Pro specifically interacts with maize chloroplast precursor of ferredoxin-5

Y. CHENG (3), Z. Liu (1), J. Xu (2), T. Zhou (1), Z. Fan (1)
(1) Department of Plant Pathology, China Agriculture University; (2) Department of Plant Science and Technology, Beijing Agricultural College; (3) Department of Pomology, China Agriculture University
Phytopathology 98:S37

Identification of interactions between viral and host proteins is essential to elucidate the molecular mechanisms underlying the viral infection process and symptom development in plants. Some dicotyledonous plant factors had been shown to interact with HC-Pro, a multifunctional protein of potyvirus, but no report has been published on the monocotyledonous host proteins that interact with HC-Pro to date. Our previous studies showed that Sugarcane mosaic virus (SCMV) (genus Potyvirus) was the main causal agent of maize dwarf mosaic disease in China, with the Beijng isolate (SCMV-BJ) from maize (Zea mays) being the prevalent strain. Thus the HC-Pro encoded by SCMV-BJ was used as a bait to screen maize cDNA library for the identification of interacting protein(s) by yeast two-hybrid system (YTHS). Our results showed that the chloroplast precursor of maize ferredoxin-5 (Fd V) inter-

acted with HC-Pro in yeast, Nicotiana benthamiana cells and maize protoplasts. Further study showed that the transit peptide rather than the mature protein of Fd V precursor could interact with both the N-terminal (residues 1 to 100) and C-terminal parts (residues 301 to 460) of SCMV HC-Pro, but not the middle part (residues 101 to 300) of the HC-Pro; HC-Pro specifically interacts only with Fd V, but not with other maize photosynthetic type Fds (Fd I and II); SCMV-infection significantly down-regulated Fd V mRNA level while has no obvious effect on mRNA levels of Fd I and Fd II. These results indicate the possible role of Fd V during SCMV infection, and suggest that the post-translational import of Fd V into maize bundle-sheath cell (BSC) chloroplast might be disturbed by the specific interaction of HC-Pro/Fd V, which could lead to the perturbation of chloroplast structure and function.

Molecular mapping of a gene for resistance to stripe rust in spring wheat cultivar IDO377s

P. CHENG (1), X. Chen (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, USA; (2) USDA-ARS and Department of Plant Pathology, Washington State University, Pullman, WA, USA
Phytopathology 98:S38

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases on wheat worldwide. 'IDO377s', a hard white spring wheat cultivar, has been grown in the Pacific Northwest for many years because of its high level resistance to many races of the pathogen. To map and tag genes in IDO377s for stripe rust resistance, we made a cross between 'IDO377s' and 'Avocet S' (AVS), a susceptible genotype. Segregation analysis of 114 F_3 lines from AVS/IDO377s revealed that a single dominant gene conferred resistance in the greenhouse seedling tests with races of PST-43 and PST-45 of the pathogen. Bulk segregant analysis identified several resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) markers linked to the resistance gene. Amplifications of the 21 nulli-tetrasomic lines of 'Chinese Spring' with some of the RGAP markers placed the resistance gene in IDO377s on the long arm of chromosome 2B. The chromosomal location of the resistance gene was further confirmed by SSR markers specific to the chromosomal arm. Based on the chromosomal location and reaction to various races of the pathogen, the IDO377s gene is likely different from all previously reported genes for resistance to stripe rust. A total of 108 wheat breeding lines and cultivars with or without IDO377s in their pedigree were used to validate the tightly linked molecular markers and to determine polymorphisms of the marker loci in various wheat genotypes.

Spatial and temporal progression of Tomato spotted wilt in flue-cured tobacco in North Carolina

K. Cherry (1), M. MILA (1)
(1) NC State University, Raleigh, NC, USA
Phytopathology 98:S38

Tomato spotted wilt (TSW) is an economically important disease caused by tomato spotted wilt virus, which is vectored by thrips (Frankliniella spp.). The spatial distribution and temporal development of TSW were studied in naturally infested fields in 2006 and 2007. Three fields were monitored in 2006 and seven fields in 2007. Fields ranged in size from 76 × 11 meters to 137 × 10 meters. In each field TSW incidence was measured on a weekly basis. TSW temporal progression for each location was fit to logistic, Gompertz, and Weibull models. TSW spatial distribution in each location was investigated using universal kriging interpolations on TSW incidence from two different dates. The logistic model was selected as the most appropriate to describe temporal progression. The spatial pattern revealed isolated clusters, but overall it was rather random, which has been previously observed with TSW in other crop systems in southeast U.S. These findings suggest that when thrips move into a field infections, even secondary infections, occur randomly. Further work will need to be done in order to determine the source of thrips, which will help refine management practices.

Analysis of the Pythium ultimum transcriptome

F. Cheung (3), J. Lang (2), J. Hamilton (4), J. Leach (2), A. Lévesque (1), N. Tisserat (2), C. BUELL (4)
(1) Agriculture and Agri-Food, Canada; (2) Colorado State University, Ft. Collins, CO, USA; (3) J Craig Venter Institute, Rockville, MD, USA; (4) Michigan State University, East Lansing, MI, USA
Phytopathology 98:S38

Pythium is an agriculturally important genus of plant pathogens and is not understood well at the molecular, genetic, or genomic level. Pythium species are closely related to Phytophthora species, the other large oomycete genus of plant pathogens, and are ubiquitious in their geographic distribution and host rage. To gain a better understanding of its gene complement, we generated Expressed Sequence Tags (ESTs) from the transcriptome of Pythium ultimum DAOM BR144 (=ATCC 200006 = CBS 805.95) using two high throughput sequencing methods, Sanger-based chain termination sequencing and pyrosequencing-based sequencing-by-synthesis. A hybrid assembly of both Sanger- and pyrosequencing-derived ESTs resulted in 34,495 unique sequences with 8,618 sequences (25%) having similiarity to Uniprot entries and containing 1,110 sequences (3.2%) that were solely derived from Sanger sequencing alone. In the hybrid assembly, were identified 179 candidate simple sequence repeats that can be used for genotyping strains of P. ultimum. Through these two technologies, we were able to generate a robust set of transcribed sequences for Pythium. We were able to identify known sequences present in oomycetes as well as identify novel sequences. An ample number of candidate polymorphic markers were identified in the dataset providing resources for phylogenetic and diagnostic marker development for this species. On a technical level, in spite of the depth possible with 454 FLX platform, the two sequencing methodologies were complementary as each method generated sequences unique to each platform.

Role of G protein in *Coniothyrium minitans* during directional growth towards exudates of *Sclerotinia sclerotiorum* and *S. minor*

P. CHITRAMPALAM (1), B. M. Pryor (1)
(1) University of Arizona, Tucson, AZ, USA
Phytopathology 98:S38

Coniothyrium minitans is a mycoparasite of sclerotia-forming fungi including *Sclerotinia* spp. It has been shown that sclerotial exudates from *S. sclerotiorum* stimulate growth of *C. minitans* and the growth is differentially greater towards the source of the exudates. However, the molecular mechanism(s) involved in the directional growth of *C. minitans* has not been characterized. G proteins are known to be involved in a number of signaling pathways and have been shown to play a role during *Trichoderma* mycoparasitism. The objective of this study is to examine the role of G protein during directional growth of *C. minitans* towards a *Sclerotinia* stimulus. Mycelial plugs of *C. minitans* were placed in the center of 2% water agar (WA) plates containing different concentration of G protein inhibitor, Suramin (0, 100, 300 µM). A strip of WA (2.5 × 1 cm) was removed from one side of the plate approximately 2.5 cm away from the mycelial plug and filled either with WA (control) or WA containing exudates of either *S. sclerotiorum* or *S. minor*. The plates were incubated at 20°C for 10 d. Growth of *C. minitans* towards and opposite to the stimulus were measured and compared. Growth of *C. minitans* towards both *Sclerotinia* exudates was significantly greater than in the opposite direction in plates containing either 0 or 100 µM Suramin. However, no significant difference was observed in plates containing 300 uM Suramin, and the growth pattern was similar to that in control plates containing no stimulus and Suramin. There was no significant difference between the growth in plates containing WA mixed with 0, 100, or 300 uM Suramin. These results indicate that G proteins play a role in directional growth of C. minitans towards stimulus but not during normal growth.

Effect of biocontrol and chemical strategies against lettuce drop caused by *Sclerotinia sclerotiorum* and *S. minor* in desert agroecosystems

P. CHITRAMPALAM (1), T. A. Turini (2), B. M. Pryor (1)
(1) University of Arizona, Tucson, AZ, USA; (2) University of California, Davis, CA, USA
Phytopathology 98:S38

Lettuce drop caused by both *Sclerotinia sclerotiorum* and *S. minor* is one of the most destructive diseases affecting lettuce in Arizona and California. Previous studies have shown that standard application rates (2-4 lbs/A) of the biocontrol product Contans (*Coniothyrium minitans*) was effective in controlling *S. sclerotiorum*, but not *S. minor*. In this study, high application rates of Contans and two isolates of the bacterium *Paenibacillus polymyxa* (strains 095 and 011) were tested for efficacy against *Sclerotinia* spp. and compared with the fungicide Endura. Field experiments were conducted at University research stations in both Yuma, AZ, and Holtville, CA. For Contans, product rates of 2 and 10 lbs/A were each applied twice. For *P. polymyxa*, suspensions of each strain of bacteria (10^9 cfu/ml) at a rate of 1 gal/A were each applied three times. Endura was applied at manufacture's rates. Results revealed that in trials against *S. minor*, two applications of high rates of Contans (10 lb/acre) and three applications of *P. polymyxa*-011 resulted in a statistically significant increase in the number of healthy heads and yields compared to control under high disease pressure. Other biocontrol treatments (*P. polymyxa*-095, Contans 2 lb) and chemical treatment (Endura) did not provide significant disease control. In trials against *S. sclerotiorum*, both rates of Contans, *P. polymyxa*-011, and Endura resulted in a statistically significant increase in the number of healthy heads compared to control under high disease pressure. In plots containing no disease inoculum, only *P. polymyxa*-011 resulted in a statistically significant increase in yield compare to control suggests growth enhancing aspects of *P. polymyxa* on lettuce.

2R,3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6 is involved in induction of systemic tolerance to drought and high salt stresses in *Arabidopsis thaliana*

S. Cho (1), B. Cho (1), K. Yang (1), Y. KIM (1)
(1) Department of Plant Biotechnology and Biotechnology Research Institute, Chonnam National University, Gwangju 500-757, Korea
Phytopathology 98:S39

Herein, we demonstrated that root colonization of *Arabidopsis thaliana* with a rhizobacterium, *Pseudomonas chlororaphis*, induced systemic protection against pathogen infection, drought stress, and salt stresses. Reduced water loss in *P. chlororaphis* O6-colonized plants correlated with smaller stomatal aperture and a higher percentage of closed stomata. Stomatal closure and drought resistance were mediated by 2R,3R-butanediol, a volatile metabolite of *P. chlororaphis* O6, in that bacteria deficient in 2R,3R-butanediol production colonized root surfaces but did not mediate drought tolerance. Studies on Arabidopsis mutant lines indicated that 2R,3R-butanediol mediated its effects through the salicylic acid (SA), ethylene, and jasmonic acid pathways, and that induced drought tolerance and stomatal closure were dependent on ABA-1 and OST-1 kinase. Increases in free SA after drought stress of *P. chlororaphis* O6-colonized plants and after 2R,3R-butanediol treatment suggested a primary role for SA signaling in induced drought tolerance. We conclude that in our Arabidopsis model, the systemic induction of abiotic and biotic stresses by rhizobacteria involved common signaling pathways, and that the bacterial product 2R,3R-butanediol was a major factor in inducing stress responses.

RhlB, a regulon of RpfF, determines virulence of *Xanthomonas oryzae* pv. *oryzae* at low inoculum density

J. CHO (1), S. Yang (1), K. Jeong (1), K. Park (1), T. Noh (2), J. Cha (1)
(1) Dept. of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea; (2) Honam Agricultural Research Institute, RDA, Iksan 570-080, Korea
Phytopathology 98:S39

The *rpf*F gene involves in production of a diffusible signal factor (DSF) that positively regulates synthesis of pathogenicity-associated factors like extracellular polysaccharide (EPS) and extracellular enzymes. To find proteins regulated by DSF in *Xoo*, total protein profiles of the wild type strain and the *rpf*F null mutant strain were analyzed in 2D-gel electrophoresis. One of the significantly decreased proteins in the *rpf*F mutant was RhlB. RhlB is annotated as an ATP-dependent RNA helicase in *Xoo* genome whose function is unknown. Protein function search showed that RhlB belongs to DEAD-box helicases family in which a diverse proteins involved in ATP-dependent RNA unwinding, needed in a variety of cellular processes. The *rhl*B gene was inactivated by marker exchange in *Xoo* KACC10859. When the mutant was inoculated on susceptible rice cultivar with different cell density, virulence of the RhlB mutant was attenuated inoculum density at 10^5 cells/ml or lower significantly compared to wild type strain. The mutant was more sensitive to hydrogen peroxide than wild type, and complementation of the gene restored the resistance to it. These results suggest that RhlB is involved in resistance to oxidative stress and knockout of the gene make *Xoo* reduced in virulence by making the bacterium sensitive to oxidative stress in rice leaf.

A role of chitinase to exhibit antifungal activity in *Chromobacterium* sp. C61

H. Choi (2), S. Park (1), Y. KIM (2)
(1) Department of Agricultural Biology, Suncheon National University, Suncheon 540-070, Korea; (2) Environmental-friendly Agriculture Research Center, Chonnam National University, Gwangju 500-757, Korea
Phytopathology 98:S39

A biocontrol agent bacterium, *Chromobacterium* sp. C61, produces extracellular chitinases and strongly inhibits growth of phytopathogenic fungi. Previous works indicated that a gene (*chi54*) encoding a chitinase of *Chromobacterium* sp. strain C-61 was composed of 1,611 nucleotides. During site directed mutagenesis process, a mutation of nonconserved residues (T218S) was obtained, which can secrete chitinase with higher activity than wild-type. To investigate role of chitinase in strain C61, Chi54 deficient mutant was constructed through marker exchange mutagenesis by insertion of *nptII* cassette into open reading frame of Chi54. The Chi54 mutant did not produce exo-chitinase on colloidal chitin medium. To construct superior activity of chitinase strain C61, we conducted a mutation that was replaced the mutated chi54 gene to the complete *chi54* gene containing T218S of *Chromobacterium* sp. C61. Antifungal activities against *Alternaria longipes*, *Botrytis cinerea*, *Pyricularia grisea*, *Phytophthora capsici*, and *Rhizoctonia solani* were similar among wild-type strain, Chi54 mutant, and T218S mutant. These results indicate that production of chitinase in *Chromobacterium* sp. C61 may not play a key role in inhibition of phytopathogenic fungi, but growth inhibition of phytopathogenic fungi could be result of combinations of production of other secondary metabolites and other extracellular enzymes.

Genetic dissection of loci conditioning disease resistance in maize bin 8.06

C. CHUNG (2), J. Poland (1), R. Wisser (5), J. Kolkman (2), The Maize Diversity Project (4), R. Nelson (3)
(1) Dept. of Plant Breeding and Genetics, Cornell University, Ithaca, NY, USA; (2) Dept. of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA; (3) Dept. of Plant Pathology and Plant-Microbe Biology; Dept. of Plant Breeding and Genetics, Cornell University, Ithaca, NY, USA; (4) The Maize Diversity Project, NSF, USA; (5) USDA-Agricultural Research Service, Raleigh, NC, USA
Phytopathology 98:S39

Northern Leaf Blight (NLB), caused by *Exserohilum turcicum*, is one of the most important diseases affecting maize production worldwide. Bin 8.06 in the maize genome is known to be associated with resistance to NLB and several other diseases. Two qualitative resistance loci and several quantitative trait loci (QTL) for NLB resistance have been localized to this region. Evaluation of the nested association mapping (NAM) population, consisting of 5000 recombinant inbred lines developed from 25 diverse maize lines, identified a QTL in bin 8.06 (designated *qEt8.06*) as the largest-effect QTL conditioning increased incubation period and decreased disease severity of NLB. In response to a recurrent selection (RS) program for NLB, significant changes in allele frequencies also showed evidence of selection acting at markers in bin 8.06. Consistent detection of *qEt8.06* in diverse mapping populations indicates that it is widespread in maize germplasm, and that it plays a substantial role in host-pathogen interactions. To dissect the genomic complex of *qEt8.06*, near-isogenic line (NIL) pairs contrasting for this locus were developed from heterogeneous inbred families derived from S11 × DK888. To characterize the resistance spectrum of $qEt8.06_{DK888}$, NIL pairs were challenged with different races of *E. turcicum*, as well as a range of maize pathogens. Preliminary results suggest that $qEt8.06_{DK888}$ confers race-specific quantitative resistance to NLB, but not to other diseases. Trait-marker association in a population of ~200 F_9 recombinants has delimited the resistance locus to a region of ~5 Mb. High marker density in the NAM population also allowed mapping of *qEt8.06* to a 7 Mb region, overlapping and narrowing the region to ~3 Mb. Future work will focus on isolating the gene(s) underlying this major QTL.

Characterization of a RNAi associated anti-viral ribonuclease in *Nicotiana benthamiana*

J. J. CIOMPERLIK (1), R. T. Omarov (1), H. B. Scholthof (1)
(1) Texas A&M University, College Station, TX, USA
Phytopathology 98:S39

An *in vitro* active siRNA-associated ribonuclease complex has been identified in *Nicotiana benthamiana* plants infected with *Tomato bushy stunt virus* (TBSV). To determine if this represents a universal anti-viral plant-defense response, experiments were conducted to examine if *Tobacco rattle virus* (TRV) can also trigger the programming of a similar anti-viral nuclease that specifically degrades viral RNA. Following infiltration with *Agrobacterium*-borne TRV expressing a segment of the plant phytoene desaturase gene, extracts from infected *N. benthamiana* were subjected to hydroxyapatite column chromatography. Fractions containing TRV-derived siRNAs and exhibiting ribonuclease activity, indicative of an RNA-induced silencing complex (RISC), were further fractionated with Sephacryl S-200 gel filtration chromatography, and the resultant samples displayed virus-specific ribonuclease activity against TRV RNA *in vitro*. The activity was inhibited by treatment with increasing concentrations of NaCl and EDTA. These preliminary data indicate that upon infection with TRV, RISC-like nuclease activity targeting TRV RNAs is established in infected plants, with a specificity similar to that observed upon TBSV infection.

Nuclear import of *Maize fine streak virus* proteins in Drosophila S2 cells

F. M. CISNEROS (2), C. Tsai (3), E. Ammar (2), M. G. Redinbaugh (2), S. A. Hogenhout (1)
(1) John Innes Centre; (2) The Ohio State University; (3) University of California, Berkeley
Phytopathology 98:S39

Maize fine streak virus (MFSV) is a member of the genus *Nucleorhabdovirus*, family *Rhabdoviridae* and is transmitted by the leafhopper *Graminella nigrifons*. The virus replicates in both its plant host and in its insect vector. *Nucleorhabdoviruses* replicate in the nucleus and assemble at the inner nuclear membrane of their plant and insect hosts, so the nuclear import of viral proteins is critical to complete viral morphogenesis. Nuclear import of proteins is mediated by a highly conserved machinery in eukaryotes that depend on importin alpha and beta. These two components are the key to the recognition and transport of proteins carrying nuclear localization signals. The molecular mechanisms involved in nuclear import of rhabdoviral proteins remain unclear. We used in situ assays to demonstrate the role for importin alpha in nuclear import of viral proteins. Fluorescent-protein fusions of the MFSV N and P proteins were transfected into Drosophila S2 cells in which

the synthesis of importin alpha was knocked down by RNA interference. In importin alpha-depleted cells the MFSV N protein distributed throughout the cells, whereas the MFSV N protein was found only in nuclei of control cells. When MFSV N and P genes were co-expressed in importin alpha-depleted cells, both N and P proteins were found throughout cells in contrast to the nuclear localization observed in control cells. These results indicate that importin alpha is involved in nuclear import of MFSV N protein and N and P protein complex into insect cells.

***Botrytis cinerea* on snap beans; targeting blossoms for disease control**

A. C. COBB (1), H. R. Dillard (1)
(1) Cornell University, NYSAES, Geneva, NY, USA
Phytopathology 98:S40

With the loss of vinclozolin registration on snap beans, control of *B. cinerea* has become more challenging. Trials were conducted *in vitro* to elucidate timing and efficacy of fungicide applications for disease control. *B. cinerea* spores (S) were applied dry by brushing them onto detached bean blossoms (cv. Hystyle) on day 1, 4, or 7. Fungicides consisting of either thiophanate-methyl + chlorothalonil (TC) or boscalid (B) were subsequently applied on day 1, 2 or 3. After 11 days incubation, less than 24% disease occurred with treatments TC1S1, TC1S4, TC1S7, TC2S1, or B1S7. Extensive disease developed in other treatments and ranged from 40 to 100%. To achieve excellent control with boscalid, the fungicide had to precede the spore application by 6 days. In 2006, a survey was conducted where fully open snap bean flowers were collected from 84 New York fields. The blossoms were plated on Potato Dextrose Agar (PDA) amended with chloramphenicol and streptomycin (ABPDA) to determine presence of *B. cinerea* inoculum in the field. *B. cinerea* infected blossoms were present in 50 of 84 fields (59.5% of the fields had one or more blossoms with *B. cinerea*), and 195 of 1017 (19.7%) of the flowers plated yielded *B. cinerea*. Summary data for 10 years of field research trials demonstrated that boscalid, boscalid + thiophanate-methyl, iprodione, and chlorothalonil + thiophanate-methyl provided good to excellent B. cinerea control 67% or more of the times tested.

Classifying and categorizing scientific literature specific to risk assessments of transgenic crops

S. D. COHEN (1)
(1) Center for Regulatory Research, LLC
Phytopathology 98:S40

Current online literature databases index scientific publications based on keywords generated from titles and abstracts. Often these keywords are not sufficient for classification/categorization of literature when writing research reviews and risk assessments. Keyword classification and clustering were examined with two scientific literature libraries generated from searches conducted on *Bacillus thuringienisis* (Bt) and glyphosate (Gly) resistant transgenic crops in CAB abstracts, a fully searchable and indexed bibliographic database, from 1973–2007. CAB abstracts online database indexed 3,649 keywords for the Bt transgenic crops literature (1532 records) and 2,273 keywords for the Gly literature (476 records). Records were imported into Endnote X, bibliographic software, and assigned groups based on major and minor topics using RefViz, data visualization software running within Endnote X. RefViz classified the Bt literature into 38 groups and the Gly literature was assigned to 21 groups. Bt and Gly libraries were further analyzed by extracting 75 keywords from EPA's guidelines for ecological risk assessment and examining the frequency of occurrences in any of the data fields. Only 35% of these keywords were found in any of the data fields of the Bt records and only 20% of the terms were found in the Gly records. Original index keywords from the CAB abstracts database did not provide the specificity for classifying and categorizing Bt or Gly publications for risk assessment. This study suggests secondary keywords specific to ecological risk assessment will need to be assigned and cross referenced to original index keywords for ascertaining the quality/quantity of the transgenic publication collections.

Toxicity of commercial algaecides to *Phytophthora ramorum*

G. C. COLBURN (1), S. N. Jeffers (1)
(1) Department of Entomology, Soils & Plant Sciences, Clemson University, Clemson, SC, USA
Phytopathology 98:S40

Oomycetes like species of *Phytophthora* are more closely related to brown algae than they are to fungi. Therefore, commercial algaecides (with copper compounds as active ingredients) used to manage algae in natural and commercial waterways might be useful in managing *P. ramorum* in similar settings. Chlamydospores of A1 and A2 isolates of *P. ramorum* were produced on mycelia grown in clarified V8 broth; cultures were sonicated to kill hyphae and free chlamydospores. Sporangia were produced by growing isolates on V8 agar and placing agar plugs in a sterile soil-extract solution, and zoospore were released after a cold temperature shock. Chlamydospores (5×10^3 spores/ml), sporangia (2.5×10^3 sporangia/ml), and zoospores (1×10^5 spores/ml) were exposed to commercial rates of two algaecides (0.8 ppm of copper carbonate and 1.0 ppm of copper-triethanolamine + copper hydroxide) for 0, 0.5, 2, 4, 8, and 24 hr. For each treatment, propagules were washed to remove algaecides and were collected on membrane filters. Filters were inverted on PAR-V8 selective medium, and plates were placed at 20C. For both isolates, zoospores were not viable after 30 min of exposure to either algaecide. Compared to the control, viabilities of chlamydospores and sporangia of both isolates were reduced significantly at 2 and 4 hr of exposure to the algaecides; no chlamydospores or sporangia remained viable at 8 or 24 hr of exposure. Consequently, algaecides have potential to manage *P. ramorum* in natural and commercial waterways.

Fungicide resistance of *Erysiphe necator* in the U.S. Mid-Atlantic region

J. F. COLCOL (1), A. B. Baudoin (1)
(1) Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
Phytopathology 98:S40

Fungicide resistance of fungal plant pathogens is a critically important problem for companies that develop fungicides and for growers. Powdery mildew (PM, *Erysiphe necator* Schwein.) is one of the most important diseases of grapes in the Mid-Atlantic region of the USA. Since common cultivars are very susceptible to PM, it must be controlled by regular application of fungicides. Some of the most effective fungicides (e.g., strobilurins and ergosterol biosynthesis inhibitors), however, have a moderate or high risk of resistance development. One hundred-four PM isolates from 31 Mid-Atlantic vineyards were tested by bioassay for resistance to azoxystrobin, boscalid, quinoxyfen, and several ergosterol biosynthesis inhibitors (EBIs): fenarimol, myclobutanil, tebucanozole, triadimefon, and triflumizole. All PM isolates were sensitive to boscalid and quinoxyfen and the majority of the isolates were resistant to azoxystrobin. Most of the PM isolates exhibited reduced sensitivity to EBIs. The average EC_{50} was highest for tebuconazole, followed by, in decreasing order: triflumizole, myclobutanil, triadimefon, and fenarimol. These fungicides differ in intrinsic activity, which is reflected in their maximum label rates; the EC_{50} expressed as a percentage of the maximum label rate was highest for tebuconazole, followed by, in decreasing order: myclobutanil, triflumizole, fenarimol, and triadimefon. The sensitivity to triadimefon was unanticipated, since previous reports suggested that it had the most serious resistance problem among EBIs. However, it has in all probability received little use in recent years and has recently been discontinued from use on grapes. The results suggest that fenarimol would provide the best PM control of the currently available EBIs. The most sensitive isolates have very little or no ability to grow on grape leaf tissue treated with 0.3 ppm of tebuconazole or myclobutanil, whereas the least sensitive isolates grew well on leaf tissue treated with more than 30 times as much of these fungicides.

Using the universal language of Gene Ontology to annotate gene products involved in the interactions between microbes and their hosts

C. W. COLLMER (8), T. Torto-Alalibo (7), M. Lindeberg (1), M. Chibucos (7), M. Gwinn-Giglio (5), B. Biehl (6), A. Ireland (2), J. Lomax (2), D. Bird (4), A. Collmer (1), R. Dean (4), J. Glasner (6), L. Hannick (3), T. Mitchell (4), N. Perna (6), J. Setubal (7), O. White (5), B. M. Tyler (7)
(1) Cornell University, Ithaca, NY, USA; (2) European Bioinformatics Institute, Hinxton, UK; (3) J. Craig Venter Institute, Rockville, MD, USA; (4) North Carolina State University, Raleigh, NC, USA; (5) University of Maryland School of Medicine, Baltimore, MD, USA; (6) University of Wisconsin, Madison, WI, USA; (7) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA; (8) Wells College, Aurora, NY, USA
Phytopathology 98:S40

Advances in genomics and pathobiology increasingly point to underlying similarities in the way that pathogens in diverse classes interact with plants. The Gene Ontology (GO) provides a controlled vocabulary for describing gene products involved in pathogenesis that universally applies to all pathogens and enables rich annotation and comparison of pathogen genomes based on gene function. The GO vocabulary describes the molecular activities of gene products, their locations in cells, and the biological processes they serve, and it provides codes for the evidence supporting term assignment. Through a multi-institutional collaboration, the Plant-Associated Microbe Gene Ontology consortium (PAMGO) is expanding the work of the global GO Consortium and has so far developed over 500 terms for specific processes in the interactions between microbes (prokaryote and eukaryote) and their hosts (plant and animal) in relationships ranging from mutualism to pathogenesis. GO terms are being used to annotate the genomes of selected plant pathogens, including the bacteria *Agrobacterium tumefaciens*, *Dickeya dadantii*, and *Pseudomonas syringae*; the oomycetes *Phytophthora sojae* and *P. ramorum*; the fungus *Magnaporthe oryzae*; and the nematode *Meloidogyne hapla*. One representative benefit is that GO annotation highlights similarities in the functions of effector proteins delivered into plant cells by bacteria and

oomycetes. PAMGO is supported by grants from the NRI/CSREES and the NSF.

Soil temperatures solarization in greenhouses in Corrientes, Argentina
M. COLOMBO (1), V. Obregón (1)
(1) EEA INTA Bella Vista, Corrientes, Argentina
Phytopathology 98:S41

Among vegetables to be consumed in fresh as tomato and pepper occupy the largest production area in the province of Corrientes, Argentina. Its economic importance takes root in high demand in national markets. In order to reduce root pathogens people used expensive soil fumigants which affect the ecological balance of soil and environment and were not successful. In Corrientes, soil solarization has been developed to control Phytophthora capsici in greenhouse and at the time different variants were consider. Temperatures above 37°C cause damage on the fluidity of cell membranes and inactivating the enzyme system of pathogens and pests of plants. In this study, soil temperatures during solarization in the greenhouse were determined in three treatments: 1) greenhouse soil completely covered with 50µ polyethylene sheets (S), 2) greenhouse soil furrow of 1 m × 0,30 m covered with 50µ polyethylene sheets (L) and, 3) close greenhouse with its soil without polyethylene sheets (T). These treatments were compared to outdoors (I). Ninety-six daily temperature values were averaged using a temperature device sensor (TEMPLOGGER-Cavadevices) at 20-cm, 40-cm and 70-cm soil depths. Treatment S had 905, 1059 and 935 hours, treatment L had 940, 990 and 868 hours and treatment T accumulates 540, 340 and 141 hours above 37°C respectively. In outdoors had not recorded values of this magnitude. More than two daily hours of lethal temperature were obtained in Corrientes as essential conditions for the control of soil pathogens.

Nematode communities and their relationships to soilborne pathogens in peanuts
K. N. CONNER (1), R. N. Huettel (1)
(1) Auburn University, Auburn, AL
Phytopathology 98:S41

There is a complex biotic structure within agronomic soils that affects plant health. Some communities of soil organisms can lead to suppression of soilborne diseases. Understanding soil suppression requires understanding the soil microbial community composition, including interactions between the populations. A system of particular interest is a peanut rotation system with a focus on *Aspergillus flavus*, the fungus responsible for aflatoxin problems. We are currently analyzing relationships among bacterial, fungal and nematode populations and their roles in subsequent alleviation of plant disease and mycotoxin contamination. These populations include free-living nematodes that can have both direct and indirect effects on soil nutrition and also affect other soil organisms. ARISA (automated ribosomal intergenic spacer analysis) is being used to fingerprint bacterial and fungal populations; however no universal primers are available for nematodes using this analysis. A DGGE (denaturing gradient gel electrophoresis) protocol is being set up to generate genetic profiles of nematodes that can then be correlated to the bacterial/ fungal relationships in this system. By utilizing this technique, we can combine the information already obtained through use of ARISA and be able to monitor molecular profiles of all soilborne organisms of interest under different crop rotations and correlate these results to aflatoxin contamination.

Automating the assessment of citrus canker symptoms with image analysis
A. Z. Cook (1), C. H. BOCK (3), P. E. Parker (1), T. R. Gottwald (2)
(1) USDA-APHIS-PPQ, Edinburg, TX, USA; (2) USDA-ARS, Ft. Pierce, FL, USA; (3) USDA-ARS/University of FL, Ft. Pierce, FL, USA
Phytopathology 98:S41

Citrus canker (CC, caused by *Xanthomonas citri* subsp. *citri*) is a serious disease of citrus in Florida and other citrus-growing regions. Severity of symptoms can be estimated by visual rating, but there is inter- and intra-rater variation. Automated image analysis (IA) may offer a way of reducing some of the errors in accuracy and precision. Actual disease was measured on individual leaves in 4 data sets of digital leaf images (with 65, 22, 123 and 200 images respectively, actual severity range 0–59%). The leaf images were assessed by three raters (VRs) and by automation with IA (Assess, APS, St Paul, MN). The groups of leaves varied in image quality (leaf quality, lighting, reflection, focus and background uniformity). Group 1 and 4 had the best quality and were most consistent. Group 2 and 3 were poorer quality. Regression analysis showed that group 1 and 4 were assessed with greatest accuracy and precision by VRs (r^2 = 0.86–0.94 and 0.78–0.84, respectively). VRs performed less well on group 2 and 3 (r^2 = 0.57–81 and 0.65–0.77, respectively). Automated IA of groups 1 (r^2 = 0.87) and 4 (r^2 = 0.82) was more accurate and precise compared to group 2 (r^2 = 0.33) and 3 (r^2 = 0.66). Whether assessed by VRs or measured by IA the data were heteroscedastic with respect to actual severity. Automation of IA can provide estimates of severity within the range assessed by VRs - provided image quality is good and uniform. Advantages of IA can include time saving and reduced error.

Comparative analysis of transcripts associated to all-stage resistance and high-temperature adult-plant resistance to stripe rust in wheat
T. E. CORAM (3), X. Huang (2), M. L. Settles (1), G. Zhan (2), X. Chen (3)
(1) Department of Molecular Biosciences, Washington State University, Pullman, WA, USA; (2) Department of Plant Pathology, Washington State University, Pullman WA, USA; (3) USDA-ARS and Department of Plant Pathology, Washington State University, Pullman, WA, USA
Phytopathology 98:S41

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a destructive disease of wheat worldwide. Genetic resistance is the preferred method for controlling stripe rust, of which two major types are race-specific and race non-specific resistance. Race-specific resistance includes the qualitatively inherited all-stage resistance, controlled by single major resistance (R) genes. Conversely, high-temperature adult-plant (HTAP) resistance is race non-specific, inherited quantitatively, and is durable. Previously, we characterized the gene expression signatures involved in *Yr5*-controlled all-stage resistance and *Yr39*-controlled HTAP resistance using the Affymetrix Wheat GeneChip. For this study, we designed and constructed custom oligonucleotide microarrays containing probes for the 116 and 207 transcripts that we had found important for the *Yr5* and *Yr39* resistance responses, respectively. We used this custom microarray to profile the resistance responses of eight wheat genotypes with all-stage resistance (*Yr1*, *Yr5*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, and *Yr17*) and five genotypes with HTAP resistance (*Yr18*, *Yr29*, *Yr36*, *Yr39*, and the HTAP resistance gene in the *Yr8* line). The aim of this analysis was to identify common and unique gene expression signatures involved in the two types of resistance, which were used to infer information regarding the general pathways involved in all-stage resistance and HTAP resistance.

Characterization of a *Pantoea stewartii* TTSS gene required for persistence in its flea beetle vector
V. R. CORREA (3), D. R. Majerczak (5), E. Ammar (2), M. Merighi (5), D. L. Coplin (5), R. C. Pratt (3), M. G. Redinbaugh (4), S. A. Hogenhout (1)
(1) Dept. of Entomology, OARDC/The Ohio State University, USA & Dept. of Disease and Stress Biology, John Innes Centre, Norwich, UK; (2) Dept. of Entomology, OARDC/The Ohio State University, USA; (3) Dept. of Horticulture and Crop Science, OARDC/The Ohio State University, USA; (4) Dept. of Plant Pathology, OARDC/The Ohio State University & USDA, ARS Corn and Soybean Research, Wooster, OH, USA; (5) Dept. of Plant Pathology, OARDC/The Ohio State University, USA
Phytopathology 98:S41

Stewart's wilt and leaf blight disease of sweet corn and maize is caused by *Pantoea stewartii* subsp. *stewartii* (*Pnss*), a bacterium that is transmitted by the flea beetle, *Chaetocnema pulicaria*. Few studies have focused on the molecular basis of the interactions of *Pnss* with its vector. Genome analyses indicated that *Pnss* carries gene clusters for two type III secretion systems (TTSS). The first (TTSS-1) encodes *hrp* genes for secretion of effector proteins into plant cells. TTSS-1 effectors are required for plant infection, but no function has been assigned to TTSS-2. The purpose of this study was to determine if TTSS-2 is involved in *Pnss* colonization and persistence in its vector. Two *Pnss* wild-type strains (DM283 and DM440) and transposon insertion mutants of a *ysaN* homolog in TTSS-2 and a *sapD* homolog were tested for persistence in flea beetles after feeding on infected plant. *ysaN* is a structural gene of the TTSS and *sapD* is an ABC transporter involved with antimicrobial peptide resistance. Immunofluorescence microscopy of dissected insect organs revealed that the wild-type and *sapD* strains persisted in the beetles for at least 12 days, whereas the *ysaN* mutant strain was significantly lower at 8 to 12 days post acquisition. Bacteria persisted primarily in the lumen of the midgut and hindgut of the beetles. Viable cell counts of *Pnss* present in beetle extracts were significantly lower for the *ysaN* mutant at 8 to 10 days post acquisition relative to wild-type and the *sapD* mutants. The lack of persistence of the *ysaN* mutant suggests that TTSS-2 is involved in *Pnss* persistence in its flea beetle vector. The relative expression levels of TTSS-1 and TTSS-2 genes in plants and in insects are currently being assessed by semi-quantitative RT-PCR.

Biological control of strawberry grey mould by *Clonostachys rosea* under field conditions
L. V. Cota (2), L. A. MAFFIA (2), E. S. Mizubuti (2), P. E. Macedo (2), R. F. Antunes (1)
(1) Student; (2) Universidade Federal de Viçosa, Viçosa, MG, Brazil
Phytopathology 98:S41

Grey mould, caused by *Botrytis cinerea*, is an important strawberry disease in Brazil. As a component of our gray mould management research program, we have been evaluating pathogen biocontrol with *Clonostachys rosea*, and selected four isolates as potential antagonists to *B. cinerea*. In 2006 and 2007,

under field conditions, we compared the efficiency of a mixture of the four *C. rosea* isolates (applied once or twice a week) to a weekly spray of procymidone alternated with captan. Following the applications and up to harvest, we evaluated weekly: leaf colonization by *C. rosea* (LCCr), average number of *B. cinerea* conidiophores on leaves (CBc), incidence of gray mould on flowers (IFlower) and fruits (IFruit), incidence of latent infections on fruits (LI), and fruit production. With applications of *C. rosea* twice a week, we got higher LCCr (16.97%), smaller CBc (10.28; 78.22 in the control treatment, sprayed with water), smaller IFlower (10.02%; 50.55% in the control), and smaller IFruit (5.95%; 25.10% in the control). Yield/plot ranged between 3.49 and 3.75 kg with applications of *C. rosea* twice a week and between 1.74 and 1.91 kg in the control. LI was 20% in the control and less than 10% in the other treatments. From our results over the two years, we recommend at least two weekly applications of *C. rosea* to successfully manage gray mould in strawberry.

QoI qualitative resistance and *CYP51A1* upstream anomalies in NY populations of the apple scab pathogen *Venturia inaequalis*

K. D. COX (1), N. L. Russo (1), S. M. Villani (1), D. M. Parker (1), W. Köller (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY 14456
Phytopathology 98:S42

Apple producers in the northeastern U.S. are strongly reliant on sterol demethylation inhibitor (DMIs) and Quinone outside inhibitor (QoIs) fungicides to manage yearly epidemics of apple scab. DMI resistance in NY populations of *Venturia inaequalis* is well documented, but the mechanisms of resistance are not completely understood. Similar to what was described previously, 32 NY *V. inaequalis* isolates representing a range of DMI sensitivities had anomalous insertions containing promoters upstream of the *CYP51A1* gene. Unlike previous reports, several baseline sensitive isolates lacked inserts all together, while highly resistant isolates provided indications of larger previously uncharacterized insertions. At the range of DMI sensitivities tested, a clearer pattern for this mechanism of DMI resistance is beginning to emerge. In 2007, we detected five isolates in a western NY orchard displaying the qualitative resistance phenotype to QoI fungicides. On sequencing the target site region in the cytochrome *b* gene, we found that all five isolates had the G143A target site mutation associated with QoI qualitative resistance in Europe. The mitochondrial mutation appeared to be at a homoplastic state on QoI-amended media. However, after three successive transfers on unamended media over the course of four months, two of the five isolates reverted to the wildtype genotype, raising questions as to mutation stability in the absence of selective pressure.

Characterization of the HrpK protein of *Pseudomonas syringae* – a putative translocator

E. P. CRABILL (1), J. R. Alfano (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 98:S42

Pseudomonas syringae pv. tomato DC3000 requires a type III protein secretion system (T3SS) for plant pathogenesis. T3SSs allow bacteria to inject or translocate type III effector proteins across the eukaryotic plasma membrane directly into host cells. In order for translocation of type III effectors to occur, the bacterium must form an open conduit through the plasma membrane of eukaryotic cells that is continuous and very near the tip of the T3SS appendage (known as the Hrp pilus) through which type III effectors travel. The proteins that function together for translocation make up a complex known as the translocon. Proteins associated with translocons, mainly from animal pathogens, have been shown to form pores in lipid bilayers. Because the plant cell wall acts as an additional barrier, the translocon in plant pathogens is likely very different from those of animal pathogens. The *P. syringae* HrpK protein is a putative translocator with sequence similarities to other putative translocators in plant pathogens. HrpK is a type III-secreted protein with at least one predicted transmembrane domain. In addition, *hrpK* mutants have a reduced ability to grow and cause disease in *Arabidopsis* as well as a reduced induction of the hypersensitive response (HR) in tobacco plants, a programmed cell death response associated with plant innate immunity. HrpK will be purified and used in pore-forming assays. Other experiments will characterize the role of HrpK in promoting the translocation of specific type III effectors. Members of another group of type III-secreted proteins called harpins present in *P. syringae* and other plant pathogens are also putative translocators. We have experiments planned to determine their relationship with HrpK. These assays along with other biological assays will help to further characterize the *P. syringae* translocon, an essential element of its pathogenicity.

Distribution of curtoviruses in weeds in southern New Mexico

R. CREAMER (1)
(1) New Mexico State University, Las Cruces, NM
Phytopathology 98:S42

Curtoviruses infect a large range of dicotyledonous plants. Weedy plants serve as reservoir hosts for both the viruses and their leafhopper vectors. To better understand the distribution of curtoviruses in southern New Mexico, a three year survey of curtovirus infection of weeds was conducted. Plants were collected from weeds at the margins of chile fields bimonthly from January 2003 through December 2005. Leaves were assayed for curtoviruses by PCR using two primer sets that amplify portions of the coat protein and rep genes, and the amplicons sequenced. During the three year period a low level of infected weeds was detected; 3.6% (68 infected/1881 tested) in 2003, 1.1% (17/1574) in 2004 and 1.0% (14/1510) in 2005. As expected, sequence variability within the coat protein genes from the samples was very low. Sequences of the rep region were used to determine curtovirus identity. In 2003 and 2004, three curtovirus types were identified in the infected plants. In 2003, 14% contained *Beet severe curly top virus* (BSCTV), 32% *Beet mild curly top virus* (BMCTV), and 46% contained a novel curtovirus first identified in pepper in 2001. In 2004, the pepper curtovirus made up 86% of the infected plants, while BMCTV and BSCTV each made up only 7% on the infections. This represents a gradual decrease since 2001 in BMCTV and BSCTV and corresponding increase in the incidence of the pepper curtovirus. Interestingly, the sequence variability in the rep region of the pepper curtovirus was very low compared to that found from BMCTV and BSCTV. These results suggest that the distribution of curtoviruses in southern New Mexico changes annually.

Survey of viral diseases of tomato in the southern region of Puerto Rico

N. Crespo (1), L. I. RIVERA (1), A. L. Gonzalez (1)
(1) UPR-RUM, Mayaguez, Puerto Rico, USA
Phytopathology 98:S42

Tomatoes are among the most important economic vegetables on the island of Puerto Rico. For the year 2005–06, Puerto Rico produced 20,304.9 metric tons and contributed $11.7 million to the island's annual gross agricultural income. Tomatoes are affected by a number of viral diseases that reduced their development and yield. Few studies have addressed the situation of viruses affecting tomatoes in Puerto Rico. The purpose of this study was to identify through immunostrips and enzyme-linked immunosorbent assay (ELISA) viruses present in symptomatic and asymptomatic tomato samples from one month old plants until harvested. To accomplish this objective, 225 samples were collected from different locations, at two experimental plots established at the University of Puerto Rico's Agricultural Experiment Station in Juana Díaz and twelve commercial plots at a tomato farm in Santa Isabel, P.R. Five viruses were tested at these two sites: *Tomato yellow leaf curl virus* (TYLCV), *Tomato chlorotic spot virus* (TCSV), *Tobacco mosaic virus* (TMV) and *Tomato spotted wilt virus* (TSWV). Eighty-eight percent and 73.5% of the samples were positive for TSWV and TMV, respectively Three different surveys were conducted at the commercial farm for TYLCV, examining 1, 3 and 6-months-old plants and were detected in 30, 0 and 12% of the samples for the first, second and third surveys, respectively. TCSV was found in 20% of the samples. The results of the viruses do not concord with the numbers of vectors in the fields.

Evaluation of alternative fungicides for organic apple production in Vermont

M. L. CROMWELL (1), L. P. Berkett (1), T. Ashikaga (1), H. M. Darby (1), T. L. Bradshaw (1), S. L. Kingsley-Richards (1)
(1) University of Vermont, Burlington, VT, USA
Phytopathology 98:S42

The objective of this trial was to compare the efficiency of potassium bicarbonate, neem oil, and *Bacillus subtilis* to a standard organic lime sulfur/sulfur fungicide program and a non-sprayed treatment for control of apple scab and other fungal diseases. Treatments were applied to 'Empire' trees arranged in a completely randomized design with five single-tree replications at the University of Vermont Horticultural Research Center in South Burlington, VT. Fungicides were applied with a handgun to drip, using maximum label rates. Applications began on 26 April and continued on approximately a weekly schedule through the end of June and then every two weeks through 23 July. Data obtained, representing the first year of a two year study, were analyzed by analysis of variance and significance between means was determined by Fisher's Protected LSD Test ($P \leq 0.05$). The alternative fungicides showed some activity against foliar apple scab compared to the non-sprayed treatment, and the potassium bicarbonate and neem oil treatments had significantly less fruit scab than the non-sprayed treatment. However, the lime sulfur/sulfur treatment provided the best overall control of scab. There were significantly more necrotic leaf spots in the neem oil and potassium bicarbonate treatments compared to all other treatments. On fruit, there was a significantly greater incidence of phytotoxic burn and russetting in the lime sulfur/sulfur treatment.

Does movement of *Colletotrichum cereale* from natural grasses and cereal crops promote turfgrass anthracnose disease?
J. CROUCH (1), B. B. Clarke (1), B. I. Hillman (1)
(1) Rutgers University, New Brunswick, NJ 08901
Phytopathology 98:S43

Anthracnose disease caused by *Colletotrichum cereale* is one of the most destructive maladies of golf course turfgrasses. The fungus has also been identified from numerous natural grasses and cereal crops, although disease symptoms are rarely observed. In this research we investigated the role of ecosystem (turf, cereal crop or prairie) and the impact of natural grass/cereal strains on turf anthracnose. Genotypic signatures from 4 nuclear genes and 22 microsatellite markers were used to analyze an extensive sample from the North America, Europe and Japan. 11 major populations were identified, structured according to ecosystem type: 3 turfgrass groups, 7 prairie/cereal groups and one diverse group comprised of both turf and non-turf isolates. The turfgrass populations were further defined according to host species: two groups almost entirely limited to *Poa annua*, the third to *Agrostis stolonifera*. In cereal/prairie populations, a similar pattern was observed, dividing wheat and oat isolates into discrete groups. Extreme differentiation between locally-adapted populations suggests asymptomatic grasses are unlikely reservoirs of infectious disease particles that could serve to fuel disease in turf. But gene flow between the generalist founder population and specialized genotypes provides a mechanism for genetic exchange between otherwise isolated populations. These findings demonstrate that while disease occurrence and spread is currently localized to the turfgrass environment, introgression between *C. cereale* ecotypes can lead to the expansion of anthracnose disease into new ecosystems.

Prevalence of frogeye leaf spot caused by *Cercospora sojina* in Ohio
C. CRUZ (1), A. E. Dorrance (1)
(1) The Ohio State University, OARDC, Wooster, OH
Phytopathology 98:S43

The severity and prevalence of frogeye leaf spot (FLS), a common disease of soybean in the southern U.S., has increased in Ohio during the previous 3 years. The objectives of this study were to determine: 1) if seed or soybean residue is the primary source of inoculum in the spring; and 2) if there is a shift in optimum temperature for growth of *C. sojina*. Soybean seed harvested from infected plants during 2006 were planted. Flats with 75 seed each were placed under mist for 30 days or in a greenhouse and watered twice per day. No leaf spots of *C. sojina* developed on any of the seedlings. In October 2007 stems and leaves with FLS were placed on the soil surface at two locations in Ohio. Conidia of *C. sojina* were detected from these stems and leaves recovered on January 2008. Fifty *C. sojina* isolates collected from 22 Ohio counties were compared to an isolate from southern Illinois. Mycelial growth at 17, 21, 25, 28, and 32°C was measured 6, 14, 22, and 30 days after plating in 2 separate experiments. There were significant differences in mycelial growth among the isolates after 30 days. These preliminary results indicate that isolates have a wide range of optimum temperatures and that this fungus may be overwintering on soybean residue in Ohio.

Wheat virus resistance via interference RNA
L. F. CRUZ (2), J. P. Fellers (3), H. N. Trick (1)
(1) Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA; (2) Kansas State University Manhattan, KS 66502; (3) USDA-ARS Plant Science and Entomology Research Unit, Dept. of Plant Pathology, Kansas State University, Manhattan, KS
Phytopathology 98:S43

Significant yield losses in Wheat (*Triticum aesativum* L) occur throughout the major wheat producing countries of the world due to viral diseases. Wheat Streak Mosaic Virus (WSMV) and Triticum Mosaic Virus (TriMV), a newly identified virus, are two of the major viruses in the Great Plains region of the United States. Cultural practices and mite vector control are the primary methods of disease management however they are not fully effective. Additionally, resistant varieties are deployed, although none of these varieties are totally effective. One biotech solution to this problem could be to use interference RNA, a phenomenon recognized as a natural defense mechanism against viral infection. To this end, partial sequences of the coat proteins (CP) of both WSMV and TriMV were amplified independently via PCR, cloned in a pENTRTOPO cloning vector and subsequently incorporated into pANDAmini expression vector. Immature embryos of the wheat cultivars 'Bobwhite' and 'Fielder' were transformed via particle bombardment using the pANDAmini vectors bearing the inverted repeat of the CP sequences and pAHC20, which contains the bar gene for selection. After tissue culture, putative transformed plants have been generated. Discussed will be our results of the transformation experiments, molecular and expression analysis and future goals and milestones for this project.

Evaluation of phosphite generating materials for Black Shank control
A. S. CSINOS (1), L. Hickman (1), K. L. Mullis (1), P. Ji (1)
(1) University of Georgia, Tifton, GA, USA
Phytopathology 98:S43

Over the past decade, the use of tobacco cultivars with resistance to Race 0 of *Phytophthora parasiticia* nar *nicotianae* (Ppn) has resulted in a shift to Race 1 of the pathogen in Georgia. There is no commercial resistance available to Race 1 of Ppn and growers must rely on the use of other agronomic methods and fungicides. However, the costs of fungicides are high and there is reluctance among growers to use them. Phosphite generating materials have been reputed to elicit SAR or have direct effects on the pathogen. This study was initiated at Coastal Plain Experiment Station in Tifton, Georgia, in a black shank nursery to evaluate their activity on black shank. Kphite, Nutriphite, and Prophyte were applied at 2.1 l/ha, 2, 4, 6, 8, and 10 weeks post plant. Alliette was applied at 1.12 kg/ha at the same timing while mefenoxam was applied at 1.12 Kg ai/ha at planting and again at final cultivation. Disease levels were high with non-treated control, Kphite, Nutriphite, Prophyte, Alliette and Mefenoxam having 97, 90, 88, 82, 97 and 72 percent disease and yields of 147, 549, 569, 820, 160 and 1936 Kg/ha dry weight. Vigor was consistently high among treatments and no phytotoxicity or stunting was recorded for any treatment. Only Prophite and mefenoxam reduced disease and increased yield ($P = 0.05$) over the non-treated control.

Whole genome sequencing of the soil fungus Rhizoctonia solani AG-3
M. A. CUBETA (5), R. Dean (5), P. Bayman (9), S. Jabaji (4), S. Neate (6), P. Nolte (7), S. Tavantzis (8), T. Toda (1), R. Vilgalys (2), N. Fedorova (3), W. C. Nierman (3)
(1) Akita University; (2) Duke University; (3) J. Craig Venter Institute; (4) McGill University; (5) North Carolina State University; (6) North Dakota State University; (7) University of Idaho; (8) University of Maine; (9) University of Puerto Rico
Phytopathology 98:S43

The fungus R. solani anastomosis group 3 (AG-3) is a member of the R. solani species complex and an important pathogen of food crops in the plant family Solanaceae. A collaborative research project is currently being conducted to obtain a high quality complete genome sequence of R. solani AG-3 isolate Rhs1AP. Sanger and 454 pyrosequencing methods will be used to provide 6X and 10X coverage of the genome, respectively, followed by automated directed sequencing into the remaining gaps. Our experimental approach involves a multiple library strategy with different insert sizes to obtain maximal genome coverage and linkage of contigs, followed by assembly and annotation. Genome assembly will be validated by use of an optical restriction map. Sequencing of full-length and assembly of normalized cDNAs will be employed to augment annotation and provide authentic gene models to the annotation process. cDNA and EST libraries will also be used to analyze alternatively spliced isoforms and single nucleotide polymorphisms. The complete DNA sequence of the fungus will reveal genes associated with its ability to cause plant disease. This information will lead to better ways of managing Rhizoctonia diseases that reduce economic loses to farmers and promote increased agricultural productivity and sustainability. This project will also provide a foundation for comparative genomic studies to better understand mutualism, saprophytism, and parasitism in Rhizoctonia fungi.

Field crop residue and other potential inoculum sources for the bacterial spot pathogen in Ontario
D. A. CUPPELS (1), T. Ainsworth (1), A. Ruggi (1)
(1) Agriculture and Agri-Food Canada, London, Ontario, Canada
Phytopathology 98:S43

Bacterial spot is a major disease of Ontario field tomatoes. The primary causal agent is the Group D form of *Xanthomonas campestris* pv. *vesicatoria*, also known as *Xanthomonas gardneri*. Seeds are thought to be the major source of inoculum but it is not known if this pathogen can survive and spread through an association with weeds, volunteer tomato plants and infected crop residue. To determine overwinter survival on crop residue, we buried debris from field plants that had been infected with Group A, B, C or D strains in nylon bags at a depth of 10 cm in mid-November. The bags were recovered the following April and sampled for the pathogens using DNA probes and a semi-selective medium. The ability of Group B and D strains to survive for two or more winters on buried crop debris was determined using a similar experimental design. Over three summers, samples (foliage and roots) of various weed species, volunteer tomatoes and wheat plants were collected from fields that had been in tomatoes the previous year and had been severely infected with the pathogen. These samples were screened for bacterial spot using DNA probes and the same semi-selective medium. The presence of the pathogen in any probe-positive sample was confirmed by PCR and pathogenicity tests. Groups B, C and D survived an Ontario winter well on crop residue but could not be found after a second winter. Small Group D populations were confirmed as present on two weed species but the association appeared

random. Although the sampling was relatively small (153 samples including 32 weed species), it appears that Group C and D forms of the pathogen may not survive from one season to the next on volunteer tomatoes, wheat plants or the weeds commonly found near Ontario tomato fields.

Identification of a chemosensory signal transduction system in *Xylella fastidiosa* associated with twitching motility and biofilm formation

L. Cursino (1), Y. Li (1), L. De La Fuente (1), C. Galvani (1), P. A. ZAINI (1), P. Mowery (2), H. C. Hoch (1), T. J. Burr (1)
(1) Cornell University, Geneva, NY, USA; (2) Hobart and William Smith Colleges, Geneva, NY, USA
Phytopathology 98:S44

Xylella fastidiosa is an important phytopathogenic bacterium that causes many serious plant diseases including Pierce's disease of grapevines. Disease manifestation by *X. fastidiosa* is associated with the expression of several factors including type IV pili that are required for twitching motility and efficient host colonization. We identified in the genome of the Temecula strain a single chemosensory signal transduction system that we propose is involved in regulation of twitching motility. Not all components of this complex signaling network, which starts from the sensing of the extracellular signal and leads to modulation of pilus retraction rate, are fully understood. We have produced mutants in different steps of the signaling cascade that have impaired motility phenotypes. These mutants are also affected in biofilm formation, and complementation of a mutant caused reversal of the phenotypes observed, supporting the notion that transition from a motile to a sessile lifestyle is coordinately regulated within the bacterial community. Understanding the molecular mechanisms underlying these phenomena will provide insight into how to control bacterial motility and biofilm formation *in planta*.

Structural and functional analysis of the rice blast fungus avirulence gene *AVR-Pita*

Y. DAI (1), Y. Jia (2), X. Wang (5), Y. Wang (4), F. N. Lee (3), J. C. Correll (1)
(1) Department of Plant Pathology, University of Arkansas, Fayetteville, AR; (2) USDA-ARS Dale Bumpers National Rice Research Center; (3) University of Arkansas, Rice Research and Extension Center, Stuttgart, AR; (4) Zhejiang Academy for Agricultural Science, P. R. China; (5) Zhejiang University, PR China
Phytopathology 98:S44

The *AVR-Pita* gene in *Magnaporthe oryzae* determines efficacy of resistance stability provided by a major resistance gene *Pi-ta* in rice. *AVR-Pita* encodes a predicted secreted metalloprotease that appears to interact with the Pi-ta protein, a cytoplasmic NBS-LRR protein, directly triggering resistance. A study was conducted to determine how structure change of the *AVR-Pita* gene impacts the effectiveness of resistance. A total of 100 isolates from the U.S. and a few other rice production areas in the world have been used for sequence analysis and pathogenicity assays. To date, the 5' leader, open reading frame and 3' trailer of *AVR-Pita* (1086 bp) have been sequenced in 70 isolates. An open reading frame encoding a protein with 224 amino acids were identified with a few amino acid substitutions or additions in all avirulent isolates. Insertion of two nucleotides was identified in the first exon leading to frame shifts in 5 virulent isolates collected in China. Four major clades were identified by nucleotide and amino acid phylogenetic analysis. Overall, sequence variation was observed throughout the 1086 bp region. More sequences were identified that lead to amino acid substitutions than silent mutations in the exons. Consistently, the corresponding fragment of *AVR-Pita* was not amplified by PCR, except one virulent isolate 60/1-5. Progress in sequencing analysis of the promoter region in 60/1-5, pathogenicity assays of a set of fungal isolates from U.S., and functional determination of *AVR-Pita* using fungal transformation will be presented.

Identification and analysis of viral sequences in peanut (*Arachis hypogaea* L.) expressed sequence tags

P. M. DANG (1), B. Guo (1), B. T. Scully (1)
(1) USDA-ARS
Phytopathology 98:S44

Crop plants grown in the field have been naturally infected with different viruses resulting in economic yield loss. In peanut, *Tomato spotted wilt Tospovirus* (TSWV), causes plant disease in major production area such as Southeastern United States. The objectives of this study were to investigate peanut sequences of expressed sequence tags (ESTs) for viral nucleotide sequences, to identify possible new viruses, and to develop control strategies of potential viral diseases. We have sequenced a total of 44,064 clones from 10 peanut cDNA libraries, derived from developing seeds at three reproduction stages (R5, R6 and R7) and from leaf tissues of a resistant and a susceptible cultivated peanuts, "Tifrunner" (a runner type, resistant to TSWV) and "GT-C20" (a Spanish type, susceptible to TSWV). We investigated the extent of viral sequences in these peanut ESTs and detected the sequences of *Peanut mottle virus* (PMV), *Peanut stripe virus* (PStV), *Tomato spotted wilt virus* (TSWV), and a potential Tobamovirus. A total of 942 sequences were identified matching viral sequences in the GenBank to PMV (606), PStV (330), and TSWV (6). We identified one peanut sequence with homologue to a pea (Pisum sativum) plant-specific Potyvirus VPg-interacting protein (PVIP) and two tobamovirus related plant proteins, a tobamovirus multiplication protein 3 (TOM3) and a Tobacco mosaic virus (TMV) helicase domain-binding protein, with homologues to *Arabidopsis thaliana*. These host proteins are essential for viral multiplication and movement from cell to cell in host plants. The presence of tobamovirus plant-responsive proteins suggests that peanut may be a host for tobamovirus. Further study of peanut putative PVIP, TOM3 and TMV helicase domain-binding proteins will enable the development of new control strategies.

Does our teaching impact the affective domain of our students?

C. J. D'ARCY (1), D. M. Eastburn (1)
(1) University of Illinois, Urbana, IL, USA
Phytopathology 98:S44

Most assessment of student learning focuses on the cognitive domain - have students mastered course content? However, many instructors also set goals related to the two other learning domains: the psychomotor domain, which includes mastery of skills, and the affective domain, which includes attitudes and values. One of the goals of our general education plant pathology course is to influence students' attitudes about agriculture and related issues. To determine whether this goal is being achieved, in Fall 2007 and Spring 2008 students completed identical 10-question opinion surveys on the first and last days of class. Questions on the importance of agriculture, controversial agricultural issues (environmental impact, food prices, genetic modification, pesticides), and the impact of agriculture and plant pathology on their daily lives were included. Only data from students who completed both surveys were used in the analyses. Pairwise comparison t-tests and chi square tests with LSD mean separation analyses gave the same results for 56 students in Fall 2007. Student concerns about eating genetically modified foodstuffs and pesticide residues on foods were significantly lower at the end of the semester than at the beginning. Significant increases occurred in student ratings of the importance of agriculture and plant pathology in their daily lives, and of the need to educate every citizen about agricultural issues. Student opinions on some issues, such as the price of food and the environmental impact of agriculture, remained unchanged. The results indicate that a general education plant pathology course can have a significant impact on students' opinions of agriculture and its role in their daily lives.

Sequence polymorphisms confer differential allele regulation of germin-like protein gene family members associated with rice blast QTL

R. M. DAVIDSON (1), P. Manosalva (3), C. Vera Cruz (2), H. Leung (2), J. E. Leach (1)
(1) Colorado State University, Fort Collins, CO, USA; (2) International Rice Research Institute, Manila, Philippines; (3) Kansas State University, Manhattan, KS, USA, Colorado State University, Fort Collins, CO, USA
Phytopathology 98:S44

Broad-spectrum genetic disease resistance is controlled by quantitative trait loci (QTL) in some rice populations. Defense response (DR) genes were associated with QTL conferring resistance against diverse populations of the rice blast pathogen, *Magnaporthe oryzae* (*Mo*) in three rice mapping populations. Oxalate oxidase-like genes (also known as germin-like proteins; *OsGLP*) which co-localized with a blast resistance QTL on chromosome (chr) 8 were the major contributors of phenotypic variance for diseased leaf area. We predicted 12 highly related *OsGLP* gene family members in the chr 8 QTL region and compared the *OsGLP* alleles in two resistant (+chr8 QTL) and two susceptible (-chr8 QTL) parental rice lines. Based on coding and promoter sequences, the genes were classified into two germin subfamily groups (OsGER3 and OsGER4). Many *OsGLP* were constitutively expressed and transiently induced by both wounding and *Mo* infection in all cultivars. Sequence polymorphisms, including an 858bp promoter insertion in the resistant donor allele of *OsGLP8-6*, were linked to observed differential expression patterns between resistant and susceptible cultivars. Our evidence suggests that multiple *OsGLP* are involved in rice defense response. Variations among cultivars in promoter and/or coding regions in the *OsGLP* gene cluster, which were originally identified as polymorphic molecular markers, are distinguishing features of resistant vs. susceptible alleles.

Dynamic genome architecture and the emergence of the phytoplasma clade

R. E. DAVIS (2), R. Jomantiene (1), Y. Zhao (2), W. Wei (2)
(1) Institute of Botany, Vilnius, Lithuania; (2) Molecular Plant Pathology Laboratory, Agricultural Research Service-USDA, Beltsville, MD, USA
Phytopathology 98:S44

Phylogenetic analyses indicating that phytoplasmas descended from an acholeplasma-like ancestor leave open the possibility that the phytoplasma

clade emerged in a "sudden" event whose traces remain in extant phytoplasma genomes. Yet, events giving rise to the origin of the first phytoplasma from its progenitor remain obscure. We suggest that footprints of events involved in the origin of phytoplasmas are present in the form of repeated genes clustered in genomic regions or islands that exhibit striking mosaicism and contain remnants from repeated and targeted mobile element attack. Tandemly repeated sequence stretches, each representing the proposed mobile genetic element, contained putatively complete genes and decayed genes (pseudogenes). Collectively, each group of tandemly arrayed remnants of mobile element formed a mosaic structure, termed a Sequence-Variable Mosaic (SVM). We interpret a hyper-variable region (HVR) within remnant mobile element genomes as a type of integron, a targeted locus for acquisition of new genes. Thus SVMs act as platforms of genome plasticity, providing loci for gene acquisition and for targeting of mobile elements to specific regions in phytoplasma chromosomes. We found that SVMs, or traces of SVMs, exist in phytoplasma strains representing phylogenetically diverse species, indicating an ancient origin in ancestral genomes. Based on these and other data, we hypothesized that SVMs formed at an early stage in evolution of the phytoplasma clade, after divergence of phytoplasmas from acholeplasmas, and must have played a pivotal role in triggering emergence of the phytoplasma clade. Our most recent findings clearly indicate that the nature of mobile element responsible for SVM formation was phage; thus, recurrent phage attack, prophage integration, recombination and gene decay originated and shaped SVMs and is at the root of evolutionary emergence of phytoplasmas (Wei et al. 2008. This Conference).

The novel *Cladosporium fulvum* effector Ecp6 contains lysine motifs that may act as carbohydrate-binding modules

R. DE JONGE (1), M. D. Bolton (1), H. van Esse (1), B. Thomma (1)
(1) Wageningen University and Research Centre, Laboratory of Phytopathology, Wageningen, The Netherlands
Phytopathology 98:S45

During infection, the tomato leaf mold pathogen *Cladosporium fulvum* secretes effectors that mediate disease establishment. A novel effector was identified by 2D-PAGE, designated Ecp6 (Extra cellular protein 6). Ecp6 contains three lysine motifs (LysM domains) that were originally found in enzymes that bind to and hydrolyze peptidoglycans present in bacterial cell walls, of which lysozyme is the best known example. More recently, LysM domains were identified in chitinases from diverse eukaryots, in legume receptor kinases that perceive nodulation factor secreted by rhizobial bacteria to establish a symbiosis, and in chitin-binding plant innate immune receptors. The involvement of LysM proteins in perception of chitin, peptidoglycan, and oligosaccharide nodulation factors supports a role in carbohydrate binding. We used a homology-based three-dimensional prediction analysis to investigate potential carbohydrate-binding sites of Ecp6-LysM domains. Based on our prediction, we hypothesize that Ecp6-LysM domains bind chitin, the Beta-(1-4)-linked *N*-acetylglucosamine oligosaccharide ($(GlcNAc)_n$) that is the major fungal cell wall constituent. To test this, we heterologously expressed *C. fulvum Ecp6* in *Pichia pastoris*. Purified Ecp6 protein will be used to determine binding specificities. We will investigate whether chitin-binding can prevent cell wall-hydrolysis by plant chitinases and can thus protect against the deleterious effects of plant chitinases. Heterologous expression of Ecp6 significantly increased the virulence of the vascular pathogen *Fusarium oxysporum* on tomato. Furthermore, by RNAi-mediated gene silencing we demonstrate that Ecp6 is instrumental for *C. fulvum* virulence on tomato.

Environmental factors affecting twitching motility, biofilm development, and aggregation by *Xylella fastidiosa*

L. DE LA FUENTE (1), P. Zaini (1), L. Cursino (1), H. Lin (2), T. Burr (1), H. Hoch (1)
(1) NYSAES, Cornell University, Geneva, NY, USA; (2) San Joaquin Valley Agricultural Science Center, USDA-ARS, Parlier, CA 93648, USA
Phytopathology 98:S45

The bacterial pathogen *Xylella fastidiosa* causes many important plant diseases in different crops such as citrus, grapes, almond and coffee. While disease symptoms expressed by this pathogen are not completely understood, it is widely accepted that blockage of xylem vessels by aggregates of the bacteria and extracellular polysaccharides is a major factor leading to disease. Work from our laboratory has provided evidence that this bacterium migrates within xylem elements by twitching motility facilitated by extension and retraction of type IV pili. This movement helps the bacterium colonize new areas of the xylem, upstream from the point of inoculation. The use of 'artificial' xylem vessels (microfluidic flow chambers) has provided information on temporal and spatial activities of the bacterium *in vitro*. This tool has been very helpful for learning new aspects of the biology of *X. fastidiosa*. Using these microfluidic chambers we have examined the effect of various environmental factors on bacterial movement and biofilm formation. Preliminary results indicate that the addition of chelating agents to the medium reduced movement of the bacteria. When a dilution of sap from a susceptible grape variety (cv. Chardonnay) was added to the microfluidic chambers, cells formed thicker and structurally different biofilms than when they were grown in PD2 media alone. Other factors such as surfactants and DNase are being studied on their influence in both biofilm formation and movement. The information obtained from these studies provides invaluable insight into the biology of the pathogen and disease development caused by *X. fastidiosa*.

Sequence comparisons between *Hosta virus X* isolates and differential infection of hosta cultivars

C. DE LA TORRE (1), D. Lewandowski (1)
(1) Department of Plant Pathology, The Ohio State University, Columbus, OH, USA
Phytopathology 98:S45

Hosta virus X (HVX) is the most economically important virus infecting hostas. HVX is mechanically transmitted and causes different symptoms including green spots, mottling, mosaic and leaf necrosis that vary according to the hosta cultivar. The use of resistant varieties and the study of HVX variability can help to minimize the spreading of this virus and the significant losses to growers. The first objective of this research was to examine sequence variability between different HVX isolates. The coat protein gene of ten Ohio HVX isolates was sequenced and compared with isolates from Minnesota (AJ517352) and Korea (AJ620114). A range of 98.9 - 100 and 99.5 - 100 percent identities at the nucleotide and amino acid level respectively, was found between all isolates. A full-length cDNA clone of Ohio isolate (H37) was constructed by overlap RT-PCR and sequenced. Comparison of the triple gene block (TGB) between H37 and AJ620114 shows 98.7, 99.7 and 98.2 nt percent identities and 96.1, 99.1 and 98.7 aa percent identities for TGB 1 (26 kDa), TGB 2 (13 kDa) and TGB 3 (8 kDa), respectively. The second objective was to screen different hosta cultivars for resistance. Twenty-four different hosta cultivars were mechanically inoculated with H37. Inoculated and non-inoculated upper leaves were assayed by DAS-ELISA at 14, 28 and 56 dpi. Twelve cultivars were systemically infected, 11 were locally infected and one cultivar was not detectably infected.

Presence and distribution of deoxynivalenol in potato tubers inoculated with *Fusarium graminearum*

J. A. DELGADO (1), P. B. Schwarz (2), J. Gillespie (2), V. Rivera-Varas (1), G. A. Secor (1)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA; (2) Department of Plant Sciences, North Dakota State University, Fargo, ND, USA
Phytopathology 98:S45

The diffusion of deoxynivalenol (DON) into apparently healthy tissue of potato tubers affected with dry rot caused by *Fusarium graminearum* (Fg) is a food safety concern for potato growers and processors, because of potential adverse effects to human health. The objective of this study was to determine the presence and distribution of DON and Fg along in potato tubers with dry rot caused by Fg. Potato tubers were inoculated with two DON-producing Fg isolates in the bud end and incubated for 3 to 7 weeks at 15°C. The evaluation consisted in Fg recovery on half strength PDA, and DON quantification by GC-MS of rotted tissue and healthy potato tuber sections distance 1, 2 and 3 cm distal from the decay margin. After 3 weeks of incubation Fg was recovered from 60, 25, 11, and 3% of the lesion and 1, 2, and 3 cm from the lesion. After 7 weeks Fg was recovered from 86, 68, 64, 53% respectively, showing a large increase of Fg into healthy tissue. But after 3 weeks of incubation, DON levels were 11.72, 0.05, < 0.04, and <0.04 ppm, in the lesion and 1, 2, 3 cm from the lesion, respectively; and after 7 weeks of incubation, DON was detected in 19.11, 0.54, <0.04, and <0.04 ppm. This shows an increase of DON in the lesion and immediately adjacent (1 cm) to the decayed area, and a lack of diffusion into additional healthy tissues with the advancing Fg hyphae. Based on this work, DON can be safely removed from tubers with dry rot by careful trimming of affected tissue.

Temperature and light effects on growth and sporulation of isolates of *Fusarium virguliforme*

P. DELGADO (1), G. Mbofung (1), L. Leandro (1)
(1) Iowa State University
Phytopathology 98:S45

The development of sudden death syndrome of soybean is known to be influenced by soil temperature, moisture, pH and light. Increased understanding of how these factors affect pathogen phenology could aid in understanding its interaction with the host as well as in defining optimal conditions for its isolation. The objective of this experiment was to determine the effect of temperature and light on growth, sporulation and chlamydospore production of four genetically different isolates of *F. virguliforme*. Inoculated PDA and SNA plates were incubated for fifteen days at 4, 10, 15, 20, 25, 30,

and 35°C. For the light experiment, incubation was done under continuous fluorescent light, 12 hour light/12 hour dark, and continuous dark. Colony diameter was measured every 3 days and sporulation and chlamydospore production was quantified after 15 days. The optimum temperature for growth was 25 C on both media, with slight variations among isolates observed on PDA. The optimum for conidiation ranged between 20 and 25°C, while chlamydospore production was highest at 30°C. On PDA, the fastest growth rate was observed under continuous light and the slowest in continuous dark. On SNA, there was variation in growth rate among isolates, with the slowest growth under continuous lighting and the fastest in the dark. Growth was faster on SNA under all lighting regimes than on PDA. Variations in colony morphology and pigmentation were observed among isolates in response to temperature and light. Sporulation and dispersal therefore may be favored at cooler temperatures, while production of survival structures may occur in warmer soils. Genetically different isolates may show variable growth and sporulation responses to environmental conditions.

How cool temperatures affect the incidence and population growth of *Erwinia amylovora* on the apple stigma

M. M. DEWDNEY (4), R. C. Seem (2), W. Kim (3), A. M. Svircev (1), H. S. Aldwinckle (2)
(1) AAFC, Vineland Station, ON, Canada; (2) Cornell University, Geneva, NY, USA; (3) Norgen Biotek Corp, Thorold, ON, Canada; (4) University of Florida, Lake Alfred, FL, USA
Phytopathology 98:S46

Cool temperature (below 17.8°C) effects on epiphytic *Erwinia amylovora* (*Ea*) populations on the apple stigma have never been fully described. Five apple cultivars Cameo, Gingergold, Royal Cortland, McIntosh, and Delicious were inoculated with three *Ea* strains (Ea 273, Ea 321, Ea 4001a) and phosphate buffer control at three continuous temperature treatments (11, 18, and 25°C). For each temperature treatment, strains were inoculated on separate branches of the same tree replicate. Blossoms were collected over a 3-day period and the populations of bacteria were quantified with real-time PCR and a Taqman probe. The data were analyzed as a repeated measures experiment as both incidence (colonization) and population data. Temperature was recorded throughout the experiment and was used to calculate cumulative degree-hours (CDH). The colonization data were fitted with CDH and the best base was determined to be 10°C. Cultivar did not have a significant effect on colonization but strain of *Ea*, day after inoculation, and the interaction cultivar × CDH did have a significant effect ($P < 0.05$). The best model for population of *Ea* on a stigma surface had significant main effects ($P < 0.05$) of CDH (base 5°C) and strain of *Ea*. Colonization appears to be the rate limiting step in the orchard spread of *Ea*, so greater attention to modeling this aspect of fire blight epidemiology is necessary for the improvement of fire blight forecasters.

A new *Pseudocercospora* species causing a serious leaf spotting and blight on *Passiflora setacea*

A. C. Dianese (2), A. M. Costa (2), J. C. DIANESE (1)
(1) Departamento de Fitopatologia, Universidade de Brasília, 70910-900 Brasília, DF, Brazil; (2) Embrapa Cerrados, Brasília, Distrito Federal, Brazil
Phytopathology 98:S46

A pathogenic *Pseudocercospora* on *Passiflora setacea* was characterized as follows: LESIONS adaxial yellow leaf spots coalescing into necrotic areas; grayish downy spots on abaxial face. IMMERSED MYCELIUM septate light brown, forming epiphyllous stromata and abaxial superficial mycelium. STROMATA (49-) 60-(102)-165 (-195) µm diam, epiphyllous, subglobose to globose, subepidermal, erumpent, *textura angularis*, brown. SUPERFICIAL MYCELIUM hypophyllous, branched, light grayish-brown; with conidiophores and conidiogenous cells; hyphae 4–6 µm diam, light brown, septate. EPIPHYLLOUS CONIDIOPHORA 7-(14)-21 (-31) × 2.5-(4)-5 µm, caespitose, sporodochial on stromata, geniculate, light brown, septate. HYPOPHYLLOUS CONIDIOPHORA lateral or terminal on the superficial hyphae. CONIDIOGENOUS CELLS 20–50 µm long, sympodial; scars inconspicuous. CONIDIA 43-(86)-129 × 3-(3.5)-5.5 µm, 5-15-septate, solitary, subhyaline to light brown. All known *Pseudocercospora* on *Passiflora* are segregated from the new species, because they lack fertile external mycelium. Additionally, in *Pseudocercospora passiflorae* only epiphyllous conidiophores, and up to 10-septate conidia are present. *Pseudocercospora stahlii*, was originally described from Puerto Rico as *Helminthosporium stahlii* F.L. Stevens in 1939, and later found in the Virgin Islands and Domenican Republic. However, most of its recent records come from 16 countries in Asia and Australia mainly affecting *P. foetida*. It is different from the species on *P. setacea* because it shows caespitose conidiophores on stromata distributed amphigenously on the leaves; and hypophyllous conidiophores occur on small stromata (20–40 µm), not on superficial mycelium as in the new species; and the mostly wide-clavate conidia in *P. stahlii* are shorter and wider with a maximum of seven septa. Finally, *P. calospilea* is different by showing smaller hypophyllous stromata (25–35 µm diam.); cylindric-obclavate smaller conidia (20-60 × 2-4 µm) just 1-7 septate. The morphological differences between the known *Pseudocercospora* on *Passiflora* qualifies the specimen studied as a new species of the genus, to be published according with the International Code of Botanical Nomenclature.

Ochratoxigenic Aspergillus species associated to grapevine in Chile

G. A. Diaz (1), R. TORRES (1), M. A. Vega (2), B. A. Latorre (1)
(1) Facultad de Agronomia, Pontificia Universidad Catolica de Chile, Santiago, Chile; (2) Facultad de Farmacia, Universidad de Concepcion, Concepcion, Chile
Phytopathology 98:S46

Ochratoxin A (OTA) is an important mycotoxin produced by species of *Aspergillus* and *Penicillium* which has been described as nephrotoxic, teratogenic and oncogenic. Previously, OTA has been detected in grapes, raisins and wines, being wine one of the most frequent source of OTA. For this reason, a tolerance of 2 ppb has been established in some countries. The objective of this study was to identify the ochratoxigenic species of *Aspergillus* associated to grapevine (*Vitis vinifera*) in the field and in wineries in the Central Valley of Chile. A total of 117 isolates were obtained from 7 of 10 vineyards and 6 of 8 wineries that were surveyed in 2005–2007. On the basis of morphological characteristics, *A. carbonarius*, *A. niger*, *A. niveus*, *A. ochraceus*, *A. paradoxus*, *A. versicolor*, and *A. wentii* were identified in field samples and *A. carbonarius* and *A. niger* were detected in the wineries. Based on ELISA's test only certain isolates of *A. carbonarius* (59%), *A. niger* (14%), *A. ochraceus* (100%), and *A. wentii* (56%) were ochratoxigenic. The production of OTA was corroborated chromatographically (HPLC and TLC). Ochratoxigenic isolates of *Aspergillus* spp. were found in cluster samples between veraison and harvest but, the highest *Aspergillus* population was obtained near harvest. The presence of ochratoxigenic *Aspergillus* associated to apparently healthy grape clusters and also found as airborne in wineries in Chile revealed that eventually may contaminate wine with OTA. To our knowledge, this is the first report of the presence of *A. carbonarius*, *A. niveus*, *A. ochraceus*, *A. paradoxus*, *A. versicolor*, and *A. wentii* on grapevine in Chile. Previously, *A. niger* was reported on table grapes in Chile.

Frequency of *Fusarium* species associated with soybean roots in Iowa

M. M. DIAZ-ARIAS (2), L. Leandro (1), G. P. Munkvold (2)
(1) Iowa State University, Dept. of Plant Pathology, Ames, IA, USA; (2) Iowa State University, Seed Science Center, Ames, IA, USA
Phytopathology 98:S46

At least twelve different species of *Fusarium* have been reported to infect soybean roots, in some cases causing significant root rot. However, the most important pathogenic species and overall impact are unclear. In order to characterize the frequency of *Fusarium* species associated with soybean roots, we collected soybean plants during growth stages V2-V3 and R1-R2 from fields in 50 Iowa counties in 2007. Roots were cultured on Nash-Snyder selective medium, and a total of ~1600 isolates was obtained. A representative sample of 420 isolates was selected for initial identification. Isolates were transferred to carnation leaf agar and potato dextrose agar, and identified to species based on cultural and morphological characteristics. Identification will be confirmed for predominant morphological species by amplification of the internal transcribed spacer (ITS) region of rDNA or other diagnostic genomic regions and comparison of sequences. We isolated 11 *Fusarium* species from soybean roots. The most frequent species were the *F. oxysporum* complex, *F. solani* complex, *F. acuminatum*, and *F. graminearum*, each recovered from 15%–25% of root samples; *F. semitectum*, *F. equiseti*, *F. sporotrichioides*, *F. proliferatum*, *F. virguliforme*, *F. subglutinans* and *F. verticillioides* were less frequent (1%–10% of root samples). Species prevalence among fields differed regionally. Representative isolates from the predominant *Fusarium* species were used for pathogenicity tests in the greenhouse.

Ecophysiological factors mitigating *in planta* survival of *P. ramorum* in California bay laurel

M. V. DILEO (1), R. M. Bostock (1), D. M. Rizzo (1)
(1) UC Davis, Department of Plant Pathology
Phytopathology 98:S46

Phytophthora ramorum, the causal agent of sudden oak death, has altered the community structure of coastal California forests by dramatically increasing the mortality rates of keystone species such as tanoak and oaks. In these ecosystems, bay laurel (*Umbellularia californica*) has been found to be the most important reservoir host for this pathogen both by supporting the majority of pathogen sporulation from ubiquitous, non-lethal foliar infections in the winter wet season and also by providing shelter during the dry, Mediterranean summer. The proportion of symptomatic bay leaves from which *P. ramorum* can be successfully isolated typically decreases during the summer. This putative loss of infection appears to occur to a greater extent within mixed-evergreen than redwood-tanoak forests. A field study was conducted during the summers of 2005, 2006 and 2007 to address these

observations and to assess associations between summer survival of *P. ramorum* within bay laurel leaves and environmental, topographic and physiological variables. Isolation success from symptomatic leaves was tracked in 50 trees within 12 sites in the Sonoma and Mayacmas mountain ranges and compared to temperature, vapor pressure deficit, elevation, insolation, canopy exposure, leaf area, leaf water potential, and lesion area data. The resulting model describes environmental and physiological constraints on the summer survival of *P. ramorum* and will assist in the development of sudden oak death risk assessments.

In vitro effect of compost teas on mycelial growth of soilborne tomato pathogens

A. Dionne (1), S. B. Koné (1), R. J. TWEDDELL (1), H. Antoun (1), T. J. Avis (1)
(1) Centre de recherche en horticulture, Université Laval, Québec, QC, Canada
Phytopathology 98:S47

Compost teas (CTs) are aqueous extracts of composts that have revealed suppressive activities toward plant pathogens. However, their efficacy in suppressing plant pathogens is variable. In order to determine the effect of compost composition on CT disease suppression, five non-aerated CTs were produced from different composts (cow, chicken, and sheep manure; shrimp and seaweed). Their suppressive activity was evaluated against four soilborne tomato (*Lycopersicon esculentum*) pathogens (*Pythium ultimum*, *Rhizoctonia solani*, *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*). Each native and sterilized (autoclaved or microfiltered) CT was incorporated into Petri dishes containing potato dextrose agar (15% v/v) and a 5-mm disc of actively growing mycelium of each of the tested pathogens was placed in the center of each dish. The radial mycelial growth was measured and compared to the water control. Results revealed that all native CTs significantly reduced the mycelial growth of all tested pathogens by 67 to 100% with CTs produced from sheep and cow manure giving the highest inhibitory effect. Autoclaved and microfiltered CTs gave variable effects on mycelial growth of the tested pathogens. Overall, this study showed that the efficacy of CTs varied according to the type of compost used in its fabrication.

Comparative Phylogenomics and Multi-gene cluster analyses of the Citrus Huanglongbing (HLB)-associated bacterium Candidatus Liberibacter

H. DODDAPANENI (4), H. Liao (1), H. Lin (3), X. Bai (1), X. Zhao (2), E. L. Civerolo (3)
(1) Guangxi Academy of Agricultural Sciences, P.R. China; (2) Guilin Citrus Research Institute, Guilin, P.R. China; (3) SJVASC, USDA-ARS, Parlier, CA, USA; (4) UC Davis/USDA-ARS, Parlier, CA, USA
Phytopathology 98:S47

Candidatus Liberibacter is an alpha-protobacterium associated with citrus Huanglongbing (HLB). It is a serious threat to citrus production world-wide. Using molecular techniques, we have successfully cloned and characterized 27 Kbp of new genomic sequences from *Ca*. Liberibacter asiaticus (Las). Here we present comparative phylogenomic analyses of the newly cloned sequences from three genomic regions. Region-1 (gene cluster) has 12 genes, Region-2 (16S-23S) has full-length 16S and 23S rRNA genes, and Region-3 (OMP region) has 11 genes. In addition, analyses of sequence variations among *Ca*. Las strains from China, Brazil, Florida and Japan, *Ca*. L. africanus (Laf) strains from South Africa and *Ca*. L. americanus (Lam) strains from Brazil for 16S region and *rpo*B gene. We identified pseudogenes, INDELs and SNPs in these cloned sequences. Phylogenetic analysis of the deduced protein sequences from the cloned regions characterizes *Candidatus* Liberibacter as a new clade in the sub-division of the alpha-proteobacteria, consistent with the 16S rRNA gene analyses. Molecular evolutionary clock estimate suggests monophyletic origin of the *Candidatus* Liberibacter species followed by speciation in conjunction with the continental drift theory. Comparative phylogenomic analysis of gene organization in *Candidatus* Liberibacter with members of the order Rhizobiales suggest overall gene structure and order conservation, albeit with minor variations including gene decay.

Functional analyses of *Aspergillus flavus* genes expressed during pathogenesis of maize

A. L. DOLEZAL (1), C. P. Woloshuk (2), G. A. Payne (1)
(1) North Carolina State University, Dept. Plant Pathology, Raleigh, NC; (2) Purdue University, Dept. Botany and Plant Pathology, West Lafayette, IN
Phytopathology 98:S47

Aspergillus flavus infects developing maize kernels and contaminates them with the carcinogen aflatoxin. To gain a better understanding of this host-parasite interaction, gene expression in the fungus and developing maize seeds was monitored during disease development. A whole genome DNA microarray containing elements representing 12,834 *A. flavus* genes and 8,895 maize genes expressed in seeds was used to measure expression during pathogenesis of field grown maize. Over 100 fungal genes and 150 maize genes were differentially expressed during pathogenesis. Four expressed fungal genes were selected for functional analysis based on predicted function or host-pathogen expression patterns. *A. flavus* homologues to PTH11, *nep1* and a Cu/Zn superoxide dismutase were included in the analysis because of their role in pathogenicity in other plant-pathogen systems. A fourth gene predicted to code for a hypothetical protein was chosen because of its coordinate expression with PTH11 and known host resistance genes. Deletion mutants for each gene were made and are being evaluated in a detached maize kernel assay for their role in seed colonization, aflatoxin production, and conidiation. Results of these studies along with a temporal profile of fungal gene expression during infection will be discussed.

Characterization of the rhizosphere bacterial community associated with *Prunus* replant disease in California

D. A. DOLL (2), L. S. Schmidt (1), D. A. Kluepfel (1), G. T. Browne (1)
(1) USDA-ARS CPGRU UC Davis, Davis, CA, USA; (2) University of California Cooperative Extension, Merced, CA, USA
Phytopathology 98:S47

Prunus replant disease (PRD) suppresses the growth of young almond and other stone fruit trees planted in soil with a recent history of their cultivation. In severe cases tree death results. The etiology of PRD is poorly understood, but it is preventable by pre-plant soil fumigation and occurs in the absence of significant populations of plant parasitic nematodes. To examine associations between soilborne bacteria and PRD we used culture-dependent and culture-independent methods (CD and CI, respectively) to characterize populations from roots of healthy trees in pre-plant fumigated soil and PRD-affected trees in non-fumigated soil. For CD, dilutions were prepared from roots and plated before and after surface disinfestation. Isolated strains of bacteria were identified by 16S rDNA sequences. For CI, total DNA was extracted from roots and adhering rhizosphere soil, bacterial 16S rDNA was amplified, cloned, and sequenced. All sequences were differentiated into operational taxonomic units (OTUs) using DOTUR and analyzed using analysis of molecular variance (AMOVA) and redundancy analysis (RDA). Based upon CHAO1 and Shannon diversity indices, greater bacterial diversity was detected using CI, but only CD revealed bacterial community shifts consistently associated with PRD. In CD communities, OTUs of *Pseudomonas* spp. and *Variovorax* spp. were negatively associated with PRD incidence, while OTUs of *Rhizobium* spp. were positively associated with the disease. Using CI, an OTU of *Streptomyces* spp. was positively associated with PRD incidence, but AMOVA and RDA did not demonstrate that this shift was consistent. Further analyses of *Pseudomonas*, *Rhizobium*, and *Streptomyces* OTUs and their involvement in PRD are justified.

Incidence of virus infections in soybean in Illinois in 2006 and 2007

L. L. DOMIER (2), H. A. Hobbs (1), G. L. Hartman (2)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL, USA; (2) USDA-ARS, Dept. Crop Sci., University of IL, Urbana, IL, USA
Phytopathology 98:S47

In Illinois in 2006 and 2007, soybean rust "sentinel" plots were sampled by sentinel scouts for virus diseases. Soybean rust sentinel plots were established in 2005 in Illinois and 25 other soybean-producing states to monitor the spread of Asian soybean rust. Leaf samples were sent to the University of Illinois for analysis by polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) for virus diseases. *Bean pod mottle virus* (BPMV) was the virus found most frequently, at 13 of 29 and 5 of 27 sentinel plots in 2006 and 2007, respectively. BPMV had an overall incidence of 2–3% in randomly collected samples both years, and 13.7% and 5.9% in samples with virus-like symptoms in 2006 and 2007, respectively. *Soybean mosaic virus* (SMV) and *Tobacco ringspot virus* (TRSV) were found at single locations, while *Alfalfa mosaic virus* (AMV), *Soybean dwarf virus* (SbDV), and *Tobacco streak virus* (TSV) were not detected in the sentinel plots. In addition, eight counties in northern and central Illinois were surveyed intensely in 2007. In each county, 30 leaf samples were collected without regard for symptoms from each of ten commercial soybean fields. Viruses were detected by PCR in total RNA extracted from bulked two-field samples for a total of 40 pooled samples. In the eight counties, BPMV was detected in 30 of 40 samples, SbDV in 17 of 40, AMV in 5 of 40, SMV in 2 of 40, and TRSV and TSV were each detected in 1 of 40 samples. These data show that BPMV remains the most prevalent virus in Illinois commercial soybean fields followed by SbDV and AMV.

Antiserum development from an outer membrane protein (*omp*) of *Candidatus* Liberibacter asiaticus

S. DONNUA (1), N. Thaveechai (1), A. Paradornuwat (1), S. Chowpongpang (1), E. Schuenzel (2), N. Schaad (2)
(1) Kasetsart University, Bangkok, Thailand; (2) USDA, Ft. Detrick, MD, USA
Phytopathology 98:S47

Huanglongbing (HLB) is considered one of the most destructive diseases of citrus in the world because it affects most citrus cultivars and causes rapid decline of infected trees. The causal agent of HLB present throughout Asia

and in parts of North and South America is *Candidatus* Liberibacter asiaticus. Two additional bacteria, L. americanus and L. africanus, are associated with HLB only in South America and Africa, respectively. We used polymerase chain reaction primers to an outer membrane protein (*omp*) to clone a 964 bp product for developing an antiserum for L. asiaticus. The product was subcloned using a pQE TriSystem® expression vector for recombinant protein expression. The expressed protein was tagged by 6xHis on the N-terminus. The 39.82 kDa product was further purified using an Ni+ NTA agarose column. SDS-PAGE analysis revealed a single band corresponding to it's molecular weight. This purified protein will be used for antibodies production and tested for specificity to L. asiaticus.

Development of a real-time PCR assay for detection of the *Raffaelea* species causing Laurel wilt disease

T. J. DREADEN (2), J. A. Smith (2), A. E. Mayfield (1)
(1) Florida DACS Division of Forestry, Gainesville, FL; (2) University of Florida, Gainesville, FL
Phytopathology 98:S48

Laurel wilt disease (LWD), caused by *Raffaelea* sp. and vectored by the redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff), is a highly destructive and exotic disease that affects redbay (*Persea borbonia* (L.) Spreng) and other plant species in the Lauraceae family. Due to the need for rapid, sensitive and accurate pathogen detection methods, a real-time PCR detection assay was developed by comparing 18s rDNA sequences from *Raffaelea* sp. (GenBank accession # EU249466) with host and pathogen sequences present in GenBank. The assay allows successful amplification of a 232 bp fragment that is not likely to be amplified in other fungi known from LWD hosts. Preliminary results from diseased red bay and avocado (*Persea americana*) samples show that the assay results in positive pathogen identification from diseased tissue but does not result in amplification from non-infected host material or other fungal species. This assay is currently being used to study pathogen colonization of host tissue and could be used for multiple applications such as quantitative studies of inoculum density and host resistance.

Efficacy of agricultural limestone amendments for suppression of Fusarium wilt in spinach seed crops in Washington State

L. J. DU TOIT (1), M. L. Derie (1), L. M. Brissey (1)
(1) Washington State University
Phytopathology 98:S48

Acid soils in western Washington are typically very conducive to Fusarium wilt in spinach seed crops, requiring 8- to 15-year rotation intervals to avoid significant losses to *Fusarium oxysporum* f. sp. *spinaciae*. The efficacy of soil amendment with agricultural limestone (97% $CaCO_3$) for suppressing spinach Fusarium wilt was assessed in field trials in 2006 and 2007 using a factorial combination of rates of limestone amendment (0 to 9.4 tons/ha in 2006, and 0 to 17.9 tons/ha in 2007) and spinach inbreds (highly susceptible and moderately-susceptible to Fusarium wilt). Spinach stand counts were not affected by limestone rate, but incidence of wilt was lower and spinach biomass significantly higher in plots with >4 tons limestone/ha compared to control plots. In 2006, 9.4 tons limestone/ha reduced wilt incidence by 45% and increased seed yield by 318% compared to non-limed plots of the susceptible inbred. For the moderately-susceptible inbred, wilt was not affected by limestone but seed yield was greatest in plots with 6.3 tons/ha compared to lower and higher rates. In 2007, seed yields did not differ among rates of limestone. Soil pH peaked within 4 weeks of limestone application, and decreased slowly thereafter for all application rates. The incidence of *Fusarium* spp. on harvested seed was low and not affected by limestone rates. However, the incidence of seedborne *Verticillium dahliae* (cause of Verticillium wilt) increased significantly with increasing rate of limestone in 2006. Soil populations of *F. oxysporum* in susceptible inbred plots were double that of plots with the moderately-susceptible inbred at harvest. Soil amendment with limestone may be a valuable tool for suppressing Fusarium wilt in spinach seed crops on acid soils.

A unique disease phenotype-'yellow shoot without blotchy mottle' was associated with a low titer of *Candidatus* Liberibacter asiaticus in Florida

Y. DUAN (2), L. Zhou (2), D. Gabriel (1), A. Weathersbee (2), T. R. Gottwald (2)
(1) Dept. of Plant Pathology, University of Florida, Gainesville, FL 32653; (2) USDA, ARS, USHRL, 2001 S. Rock Rd, Ft. Pierce, FL 34945
Phytopathology 98:S48

Citrus huanglongbing (HLB), is one of the most devastating diseases of citrus worldwide. The disease is associated with three different species of Candidatus Liberibacter: Ca. L. asiaticus, Ca. L. americanus and Ca. L. africanus. Ca. L. asiaticus (Las), first detected in Florida in 2005, has been detected from the majority of highly symptomatic HLB samples collected from commercial and residential citrus throughout Florida. The disease in Florida is transmitted by the psyllid, Diaphorina citri. Current diagnosis of HLB relies on symptoms typical of HLB, including yellow shoots (YS) and/or blotchy mottling of leaves (BML), followed by PCR confirmation using Las-specific primers. Here we report a unique disease phenotype, YS without BML in grapefruit, pummelo and sweet orange found in Florida during 2006–2008. In contrast to plants with BML symptoms, the diseased plants expressing YS only contained an apparently very low titer of Las bacterium, which often resulted in failure of detection of the bacterium using current conventional and real-time PCR protocols. We improved sensitivity of HLB detection both by improved sampling and by concentrating the bacterial DNA. The apparent bacterial titers in the diseased plants with YS only and in psyllids collected from these plants appeared hundreds of times lower than those in the diseased plants with BML and in the psyllids from them. Transmission of YS symptoms without BML was demonstrated by grafting. These differences in HLB phenotypes and associated bacterial titer suggest either Las strain variation in Florida or a possible disease complex of HLB pathogens.

Molecular characterization of a group 16SIII phytoplasma associated with decline of China-treeE (*Melia azedarach* L.) in Brazil

V. DUARTE (2), E. G. Silva (1), I. R. Hass (1), I. Bedendo (1), E. W. Kitajima (1)
(1) ESALQ, Piracicaba, SP. Brazil; (2) UFRGS, Porto Alegre, RS, Brazil
Phytopathology 98:S48

China-tree is an exotic species in Brazil, originated from Asia, used as an ornamental shade tree in the South of the country. Since 2005, plants displaying yellowing, little leaves, witches' broom and dyeback have been observed. Transmission electron microscopy detected pleomorphic bodies, 400–2000 nm in diameter, in the phloem vessels of symptomatic leaves. Total DNA from leaves was used in nested PCR performed with the universal primer pairs P1/Tint, followed by 16SrF2n/R2. Amplified product by P1/Tint was used as template in PCR with the group-specific primers pair R16(III)F2/R1 for 16SrIII group. DNA fragments amplified by reaction primed by 16SrF2n/R2 were analyzed by RFLP using the restriction enzymes *Alu*I, *Hha*I, *Hpa*II, *Kpn*I, *Mbo*I, *Mse*I, and *Rsa*I. Phytoplasma was consistently detected in all symptomatic samples by visualization of typical bands of 1.2 kb in agarose gel. DNA fragments of 0.8 kb were amplified in reactions primed by group-specific primer pair, indicating the presence of a group 16SrIII phytoplasma. RFLP analysis revealed electrophoretic profiles that permitted identify the phytoplasma as a member of group 16SrIII, subgroup B, and confirmed the results obtained with PCR carried out with group-specific primer pair. These data confirm phytoplasma as the causal agent of the decline of china-tree in Brazil, previously described by us based upon only on symptomatological aspects.

The removal of *Phakopsora pachyrhizi* urediniospores from soybean leaves by rainfall

N. S. DUFAULT (2), S. A. Isard (2), J. J. Marois (1), D. L. Wright (1)
(1) NFREC, University of Florida, Quincy, FL, USA; (2) Penn State University, University Park, PA, USA
Phytopathology 98:S48

The impact of soybean rust, *Phakopsora pachyrhizi*, on the major soybean producing regions of the United States is dependent upon viable urediniospores landing and remaining on distant host tissues. Rainfall can deposit substantial quantities of urediniospores onto soybean leaf tissue in wet and cloudy environmental conditions that are favorable for infection and adhesion. The objective of this study was to examine the effect of prolonged periods of rainfall on the removal of urediniospores from soybean leaf tissue. Spores that had been deposited by the simulated rainfall intensities of 15 and 75 mm/hr were exposed to prolonged simulated rainfall periods of 1 and 30 minutes at the same intensities. Soybean leaf samples were collected from the exposed plants, incubated for 14 days and the number of uredinia per cm squared was recorded. It was observed that significant ($P < 0.05$) proportions of spores can be removed by 1 and 30 minutes of prolonged rainfall at both intensities. Prolonged rainfall periods of 30 minutes removed 69 to 93% of the estimated spores originally deposited on the leaf tissue. These results indicate that prolonged rainfall periods following wet deposition events will significantly reduce the number of spores present on the leaf tissue. However, even though prolonged rainfall reduces the number of spores present, it did not eliminate them from the leaf tissue before adhesion could begin.

Evaluation of *Mentha arvensis* for resistance to *Verticillium dahliae* isolates from various hosts

J. K. DUNG (1), B. K. Schroeder (1), D. A. Johnson (1)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, USA
Phytopathology 98:S48

Verticillium dahliae, causal agent of Verticillium wilt (VW), is an important pathogen of cultivated mint. Resistant cultivar development is a valuable approach to manage VW. Two *Mentha arvensis* lines, "Paraguayan" and "Shivalik", were inoculated in the greenhouse with *V. dahliae* isolates from

several vegetative compatibility groups (VCG) from various hosts. *M.* x *piperita* (peppermint) and *M. spicata* (native spearmint) were used as susceptible and resistant standards, respectively. Disease severity index (DSI) ratings were recorded during the first eight weeks after inoculation; plants were then cutback and DSI ratings and yields recorded after two periods of re-growth. DSI values were significantly lower in *M. spicata* than in other cultivars when inoculated with isolates from mint (VCG2B), however DSI values and yields were significantly higher and lower, respectively, in peppermint than the other three cultivars following both re-growth periods. Higher DSI values and reduced yields were observed in plants inoculated with isolates from mint rather than with isolates from other hosts, including a VCG2B isolate from spinach, indicating variation in aggressiveness among and within VCGs of *V. dahliae*. *M. arvensis* plants exhibited high DSI values following inoculation but lower DSI values after cutback and re-growth, indicating that recovery from infection is an important consideration when determining disease resistance in perennial plants.

Characterization of a new strain of *Streptomycete* causing symptoms associated with potato common scab from Michigan soil
S. DURAIRAJ (2), J. Yin (2), R. Hammerschmidt (2), W. W. Kirk (2), D. Douches (1), J. Hao (2)
(1) Department of Crop and Soil Sciences, Michigan State University, E. Lansing, MI 48823, USA; (2) Department of Plant Pathology, Michigan State University, E. Lansing, MI 48823, USA
Phytopathology 98:S49

Potato common scab (*Streptomyces scabies*) can be caused by more than one species of *Streptomyces*. A new strain of scab causing *Streptomycete* was isolated from a Michigan potato field using dilution plating on Streptomyces selective medium. Genomic DNA was extracted using a Mo Bio extraction kit. The taxonomy of the strain was confirmed by morphological and biochemical characteristics, and analysis on 16s rRNA gene sequence. The strain formed a distinct phylogenetic line; the organism was most similar to the type strain of *Streptomyces* sp. ME02-6979.3a from the gene bank, but distinct from other known pathogenic Streptomyces species. The new strain has the genes encoding the synthetase thaxtomin and tomatinase, which are required for the pathogenicity. However, the *nec1* gene, characteristic of the Streptomyces pathogenicity island was not detected. The strain grew at low pH with a minimum of pH 4.5, and caused lesions on potato tuber slices in the preliminary assay experiments. The greenhouse experiments are in progress to further confirm the pathogenicity of the strain in the potato tubers. Further genetic analyses will be conducted to identify the pathogen.

Localization of *Acidovorax avenae* subsp. *citrulli* (Aac), the bacterial fruit blotch pathogen in naturally infested watermelon seed
B. DUTTA (1), L. L. Genzlinger (1), R. R. Walcott (1)
(1) University of Georgia, Athens
Phytopathology 98:S49

Bacterial fruit blotch (BFB) of cucurbits, caused by *Acidovorax avenae* subsp. *citrulli* (Aac) has emerged as one of the most economically important diseases of watermelon worldwide. While seed is the primary source of Aac inoculum, the fact that antimicrobial seed treatments fail to reliably eliminate the bacterium suggests that the bacteria reside under the seed coat. However, at present the exact location of Aac in naturally infested seed is unknown. Hence, the goal of this study was to investigate the localization of Aac in naturally infested watermelon seeds. Watermelon seeds were carefully dissected into perisperm-endosperm layer (a thin suberized envelope which completely encloses the cotyledon), seed coat and cotyledon and each subsection was tested for Aac by real-time PCR and plating on semiselective media. Aac was detected in perisperm-endosperm layer (PE) (10/10) and seed coat (3/10) tissues but was not detected in cotyledon tissues (0/10). To understand the effect of Aac localization on BFB seedling transmission, naturally infested watermelon seeds (n = 100 seeds) with and without seed coats and PE layers were planted and observed for BFB symptoms after 14 days. A reduction in disease transmission was observed for seeds without PE layers (40.4%) as compared to seeds with seed coats (85%) and PE layers (90%). These results suggest that Aac is located on the surface of the PE layer in naturally infested seeds.

Surface plasmon resonance (SPR) detection of potato wart fungus
J. Duy (1), L. CONNELL (1)
(1) University of Maine, Orono, ME, USA
Phytopathology 98:S49

The chytridiomycete Synchytrium endobioticum is a soil-borne pathogen that infects susceptible potato cultivars and infests the soil by releasing thick-walled sporangia which are easily transported and are viable for at least 30 years. Diseased potato plants develop wart tissue on tubers and root systems, rendering crops inedible. The pest is endemic in the Netherlands and parts of Canada; it is of prime importance to restrict the entry of the organism into uninfected regions since its spread could have substantial economic impact on the agricultural industry. Infected plots are tested periodically over 30 years to determine the presence of infectious sporangia in the soil; current testing methods require skilled, trained personnel and are labor intensive. Therefore, a quick, easy, and inexpensive method of detecting the presence of S. endobioticum is desirable. A field-compatible surface plasmon resonance (SPR) device has been constructed to address this problem, as SPR can detect real-time hybridization events. A probe layer of peptide nucleic acid (PNA) was used to identify S. endobioticum DNA, as PNA are resistant to proteases and nucleases and have a high mismatch discrimination. The probe (250 μM concentration) was covalently bound to a gold-coated Spreeta chip for 3 hours, rinsed with nanopure water to remove unbound strands, then dried before use. A solution containing a complementary S. endobioticum DNA sequence was flowed past the chip surface at concentrations of 1000 ng/mL and 10,000 ng/mL. The SPR instrument was able to detect the hybridization of the target sequence to the probe layer within 5 minutes.

"Taking it home" – a project to assess student use of class material
D. M. EASTBURN (1), C. J. D'Arcy (1)
(1) University of Illinois, Urbana, IL, USA
Phytopathology 98:S49

Do students relate what they are learning in the classroom to their daily lives? Do students "take home" knowledge, experiences, and skills gained in class and apply them or reflect on them in settings outside the classroom? If so, how, when, where, and with whom does this transfer occur? During Fall 2007 and Spring 2008, students in our general education plant pathology course wrote weekly online journal entries describing if and how they had recently related course content to their daily lives. Journal entries counted for 10% of their grade and were evaluated by a teaching assistant for relevance, completeness, organization and technical merit. Entries were private and inaccessible to the course instructor during the semester to avoid the perception that journal content could subjectively affect grades. Data from journals of selected female and male students and selected science and non-science majors were coded and analyzed for themes. Preliminary results indicate that topics directly related to a student's past and present personal experiences were "taken home" most often, and this occurred when students were reflecting on course information or were talking with friends or family members. In many cases these thoughts or discussions arose when the students were eating, preparing, or shopping for food, or when driving by or visiting agricultural settings. The topics most commonly reported tended to relate to class information presented in the form of a story, associated with a "show and tell" object, or related to a writing assignment. The results of this study will be used to modify the content and presentation of class material so that it is more likely to be "taken home" by students.

The effect of packingline impacts on susceptibility of sweetpotatoes (*Ipomoea batatas*) to *Rhizopus stolonifer*
B. A. EDMUNDS (1), G. J. Holmes (1)
(1) North Carolina State University, Raleigh, NC, USA
Phytopathology 98:S49

Methods of packing and handling sweetpotatoes are important for mitigating postharvest losses due to decay by Rhizopus soft rot (caused by the wound-obligate pathogen, *Rhizopus stolonifer*). Controlled studies were completed to understand the relationship between packingline impact forces and susceptibility to Rhizopus soft rot. Two cultivars (Hernandez and Beauregard) were injured by dropping from five heights corresponding to impact forces of 0, 10, 20, 40 and 60 G (1 G = 9.8 m/s^2). The roots were dropped onto a steel surface from two orientations: onto the root end or onto the root midsection. After dropping, the roots were dip-inoculated in a *R. stolonifer* spore suspension, stored at 14C, and rated for disease incidence after 10 days. Hernandez was significantly more susceptible to Rhizopus soft rot than Beauregard. Both cultivars showed a positive relationship between impact force and decay. In both cultivars, impacts on the root end resulted in higher decay than the same impact force on the root midsection. A separate experiment was conducted to study the multiplicative effect of a single packingline drop (height = 30.5 cm/impact force = 30 G). Results showed Hernandez to again be significantly more susceptible than Beauregard to Rhizopus soft rot. A single drop was capable of causing injuries that resulted in decay by *R. stolonifer* and multiple drops resulted in increased decay incidence. These results will be useful in developing guidelines for modifying sweetpotato packinglines to reduce losses to Rhizopus soft rot.

***Phakopsora pachyrhizi* host penetration strategy**
H. EDWARDS (1), M. R. Bonde (2)
(1) Department of Biological Sciences, Western Illinois University, Macomb, IL, USA; (2) USDA ARS FDWSRU, Frederick, MD, USA
Phytopathology 98:S49

Unlike most rusts that indirectly enter a leaf via the stomata and then penetrate mesophyll cell walls using enzymatic digestion, the soybean rust pathogen

(*Phakopsora pachyrhizi*) penetrates directly through the host epidermal cell. Thus besides the host wall, this imposes the additional barrier of breaching the host cuticle. Fungal pathogens have been shown to use either of two strategies for breaching the outer epidermal host cuticle and wall. One mechanism involves developing pressure that will puncture through cuticle and wall. The second utilizes secretion of digestive enzymes that digest both the cuticle and the wall. Understanding these penetration processes may provide useful avenues for developing resistance strategies. We show using transmission electron microscopy that the soybean rust fungus uses both mechanisms in breaching the host cuticle and host wall. Pressure is used to penetrate the cuticle and enzymes used to digest the cell wall. Penetration of the lower epidermal cell wall by the penetration hypha is via enzymatic digestion. Enzymatic digestion is also the method of penetration into mesophyll cells to form haustoria.

Incidence and diversity caulimoviruses in wild dahlia species from the Mexican highlands and the cultivated dahlias in the U.S.

S. EID (2), K. Druffel (2), D. Saar (1), H. Pappu (2)
(1) Murray State University, Murray, KY, USA; (2) Washington State University, Pullman, WA, USA
Phytopathology 98:S50

Dahlia mosaic is a serious disease affecting cultivated dahlia (*Dahlia variabilis*). In addition to *Dahlia mosaic caulimovirus* (referred to as DMV-Portland) reported previously, we characterized two new caulimoviruses (DMV-D10 and DMV-Holland) from dahlia. The relative incidence of these three viruses in wild and cultivated dahlia species was determined. A total of 249 samples were collected from cultivated dahlias (D. variabilis) from the U.S. and wild dahlias (*D. coccinea*, *D. rupicola*, *D. tenuicaulis*, and *D. sherfii*) from the highlands of Mexico. Mexico is considered as the center of diversity for *Dhalia*. These samples were tested for the three caulimoviruses using virus-specific primers and polymerase chain reaction. Virus incidence in cultivated dahlia samples collected between June to September 2007 showed that DMV-D10 was most widely prevalent (95%) followed by DMV-Holland (54%) and DMV-Portland (27%). Of the 56 samples of wild dahlia species that were tested, DMV-D10 was predominant (89%) followed by DMV-Portland (5%). Ten percent of the wild dahlia samples were free of all three viruses. Production of virus-free stock and the virus management tactics for viruses associated with dahlia mosaic should take into account the diversity of caulimoviruses extant in dahlia.

Virtual nematode specimens for teaching nematology

J. D. EISENBACK (1)
(1) Virginia Tech, Blacksburg, VA, USA
Phytopathology 98:S50

Teaching students about the morphology and taxonomy of nematodes requires numerous specimens of various kinds of nematodes that illustrate the wide diversity of the phylum. Collecting the specimens and mounting them on slides can be quite difficult, especially when numerous slides must be made on or near the day of the laboratory session. In addition, the specimens must be well preserved and lying in a nearly lateral position so that all of the necessary details can be seen. Unfortunately, prepared slides are subjected to users that may not be familiar with the light microscope, and many microscopes in student laboratories cannot compete with research grade microscopes. Fortunately, digital photographs of nematodes can take the place of preparing all of these specimens. With a research microscope using a high quality, oil immersion objective lens, a series of 3-15 photomicrographs of the entire nematode are stitched together to produce a high resolution 50-500 megapixel mosaic that substitutes for real specimens. These virtual specimens can be viewed on a computer monitor at low magnification that can be gradually increased all the way to full oil immersion magnification. These images allow each student to observe, with a high quality research grade microscope, the best specimen in the correct position. Furthermore, virtual specimens are nearly indestructible, they can be used year after year, and the students can keep these specimens on their own computers for additional study and review.

Characterization and genetic relationships of *Verticillium dahliae* populations in Lebanon

I. El Rassi (1), K. Bou Azza (1), A. T. SAAD (1), L. Hanna (1), L. Geagea (2)
(1) American University of Beirut, Beirut, Lebanon; (2) Universite St Esprit, Kaslik, Lebanon
Phytopathology 98:S50

The wilt disease caused by Verticillium species is a very serious disease affecting a wide range of host plants in Lebanon. The objectives of this study were to isolate, characterize, and speciate Verticillium strains from Lebanon using morphological characteristics and molecular techniques. Moreover, genetic diversity among these strains was tested by their vegetative compatibility, using complementary auxotrophic nitrate-nonutilizing nit mutants generated from the wild type strains. Eighty-eight Verticillium strains were isolated from naturally infected almond, olive, peach, and potato plants. Morphological characteristics of monosporic colonies of the isolates identified 81 strains as Verticillium dahliae, 4 Verticillium albo-atrum and 3 Verticillium tricorpus. The identity of five Verticillium isolates from almond, five from peach and forty-six from potato, was confirmed by ELISA tests as genus Verticillium. Genomic DNA of the fungus strains was extracted and PCR was carried out using three V. dahliae primers; DB22, DB19 and espdef01 in a ratio of 1:7:2, respectively. All ten Verticillium strains from olive, 8 strains from potato, one strain from almond and one strain from peach were confirmed as V. dahliae by the PCR molecular technique. However, three strains from almond and four strains from peach, characterized morphologically as V. dahliae, were not confirmed by the PCR test using the same primers. Different primers of V. dahliae and primers for other Verticillium species will be used to clarify this discrepancy. The eighty-eight strains tested were assigned to four vegetative compatibility groups in Lebanon.

Identification of phytoplasmas affecting greenhouse tomatoes in North America

S. Y. ELATEEK (1), M. L. Lewis Ivey (1), S. A. Miller (1)
(1) Department of Plant Pathology, Ohio State University, OARDC, 1680 Madison Ave., Wooster, OH, USA
Phytopathology 98:S50

Greenhouse tomato *Solanum lycopersicum* production has grown markedly in North America in the last 20 years, now constituting 37% of fresh market production. Several diseases have caused significant economic losses in greenhouse tomatoes, including bacterial canker and pepino mosaic virus. Recently, symptoms typical of a phytoplasma disease were observed in production greenhouses in Arizona, Texas and Mexico. Infected plants showed growth distortion, proliferation of lateral shoots, greening of flower petals, and swollen green buds that failed to set fruit. The presence of phytoplasmas in diseased tomato plants was detected using polymerase chain reaction (PCR) assays. Total genomic DNA from tomato samples from each location was amplified using nested PCR with the aster yellows-specific primers P1/P7 followed by F2n/R2. A 1.2 Kb fragment from all the samples was produced suggesting that the phytoplasma in each location is genetically related to the 16SrI (aster yellows) group. For each sample and location the 1.2 Kb PCR products was digested with five endonuclease enzymes *AluI*, *PstI*, *HhaI*, *RsaI* and *HindIII* and analyzed using restriction fragment length polymorphism (RFLP). The RFLP fingerprints from each tomato sample and location were identical to that of reference strain AY-WB, a member of the 16Sr1-B phytoplasma subgroup.

Online teaching: Engaging students through interactive discussions

S. D. ELLIS (1), M. J. Boehm (1), C. R. Curtis (1)
(1) The Ohio State University, Department of Plant Pathology, Columbus, OH, USA
Phytopathology 98:S50

Since 2001, the Department of Plant Pathology at The Ohio State University has offered an online course entitled “Social Impact of Plant Diseases in Shaping Human Society”. Our three-credit hour course is presented via Carmen, Ohio State’s version of the online course management system, Desire-to-Learn. The vast majority of students taking the class are non-science majors. Examples of topics covered in weekly lessons range from basic concepts and history of plant pathology to famine issues and bioterrorism. The average enrollment per offering is ~50 students. Similar to traditional classes, there are quizzes, assignments, and exams, but here they are submitted and managed through Carmen. A unique aspect of the class is the use of instructor facilitated discussions in which students are required to post a short reflective response to the material presented in a given lesson and also respond to each other’s postings. The instructor provides direct feedback and additional information as appropriate. The main advantage of including these discussions is that the facilitator renders a human element in an otherwise virtual experience. A major challenge is the time it takes the instructor to read, review and respond to all the postings, which is ~1,200 messages per session. The purpose of this poster is to highlight how we teach this online class and to provide an opportunity for us to interact with others who teach similar courses.

Comparison of strobilurin type fungicides to control soybean seedling pathogens

M. L. ELLIS (1), K. D. Broders (1), A. E. Dorrance (1)
(1) The Ohio State University, OARDC, Wooster, OH
Phytopathology 98:S50

Seedling diseases in soybean fields in Ohio have increased over the past decade. Most likely due to the occurrence of cool, moist soil conditions shortly after planting that delay seed germination, and favor growth of soil-

borne pathogens, such as *Phytophthora sojae*, *Pythium* spp. and *Fusarium graminearum*. In this study azoxystrobin, trifloxystrobin, and pyraclostrobin fungicides were compared for their efficacy to control six isolates of *F. graminearum*, two isolates of *P. sojae*, and 17 isolates of *Pythium*, with each isolate representing a different species. Plate assays, which measured the mycelial growth of each isolate at concentrations of 0.01 ppm, 0.1 ppm, 1 ppm, 10 ppm, and 100 ppm were completed for each of the fungicides. The results indicate that azoxystrobin and pyraclostrobin are effective in reducing growth of the 17 different *Pythium* spp. starting at 10 ppm, while trifloxystrobin showed little control even at the highest concentration. The fungicides showed no significant control in reducing mycelial growth of *P. sojae* or *F. graminearum* isolates. These results indicate that along with other cultural control methods, seed treatments, using azoxystrobin and pyraclostrobin compounds, can be effective in limiting growth of 17 different species of *Pythium* isolated from diseased soybean seedlings in Ohio.

Efficacy of fungicides against *Fusarium graminearum* isolates associated with soybean seedling diseases in Ohio

M. L. ELLIS (1), K. D. Broders (1), P. A. Paul (1), A. E. Dorrance (1)
(1) The Ohio State University, OARDC, Wooster, OH
Phytopathology 98:S51

Fusarium graminearum is an important pathogen of wheat and corn in Ohio, and more recently it has been found to be associated with diseased soybean seeds and seedlings. Six isolates of *F. graminearum* collected from infected corn, soybean, and wheat in Ohio were evaluated to determine their pathogenicity to soybean seeds, using a rolled towel assay. Twenty seeds, cultivar Sloan, were inoculated on a towel with one isolate using100 µl of a spore suspension in sterile water at concentrations of 2.5×10^2, 2.5×10^3, 2.5×10^4, or 2.5×10^5 spores/ml. The rolled towels were placed in a bucket and incubated in the dark at 18°C, 21°C, and 25°C for 7 days. All isolates were pathogenic to soybean seeds with highest disease levels with an inoculum dose of 2.5×10^4 spores/ml or higher at 25°C. This method, under optimum conditions, was then used to evaluate the efficacy of Captan, Maxim, and Apron as seed treatments to control *F. graminearum*. Based on the percent seed germination and the amount of symptom development on the roots, both Captan and Maxim significantly reduced disease development compared to Apron. This study will assist growers in determining which seed treatments are most suitable in controlling seedling diseases caused by *F. graminearum*.

The pathogenicity and phylogeny of *Fusarium oxysporum* isolates on *Coreopsis verticillata* 'Moonbeam'

W. H. ELMER (3), M. M. Jimenez-Gasco (2), M. L. Daughtrey (1)
(1) Cornell University; (2) Pennsylvania State University; (3) The CT Agr. Exp. Sta.
Phytopathology 98:S51

In the summer of 2006, Fusarium wilt of *Coreopsis verticillata* cv. Moonbeam was first reported and the causal agent was identified as *Fusarium oxysporum* (Fo). Given the recent appearance of this serious disease and the potential for widespread distribution via the horticultural trade, we investigated the host range of the pathogen. Ground millet colonized by Fo from coreopsis was used to infest potting mix (2.5 g millet/L potting mix). No wilt symptoms or reductions in dry weight were observed in 3 cultivars of aster, 8 cultivars of chrysanthemum, and one cultivar of echinacea when compared to controls. Similarly, no symptoms were observed on ageratum, basil, eggplant, gomphrena, scabiosa, and snapdragon. In contrast, symptoms of stunting, wilt, and vascular discoloration were observed on *C. verticillata* and *C. lanceolata*. These data suggest that a *forma specialis* designation may be applicable to this pathogen. To assess the level of genetic diversity in the pathogen, the tef-alpha gene genealogy from 17 Fo isolates was developed, including isolates from other *formae speciales* of Fo. All of the coreopsis isolates were closely related and placed in a single group within Clade 2 of the Fo species complex, indicating that host specialization might have occurred from a single ancestor. We propose the pathogen be called Fo f. sp. *coreopsii*.

Biological control of wilt disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by endophytic ACC-deaminase producing actinomycetes in the United Arab Emirates

K. EL-TARABILY (1), F. McKenna (2)
(1) Department of Biology, Faculty of Science, United Arab Emirates University, Al-Ain, 17551, United Arab Emirates; (2) Natural Science Center Inc., 568 Duncan Farm Road, Steele, AL 35987, USA
Phytopathology 98:S51

Forty-five endophytic streptomycete actinomycetes were obtained from surface-sterilized tomato roots and were evaluated for their potential to produce cell-wall degrading enzymes, antifungal metabolites, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and to inhibit the *in vitro* growth of *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of tomato wilt in the United Arab Emirates. The most inhibitory isolates produced antifungal metabolites, high levels of chitinase and beta-1,3-glucanase, ACC deaminase, and degraded the hyphae of *F. oxysporum* f. sp. *lycopersici in vitro* causing extensive plasmolysis and cell wall lysis. ACC deaminase-producing biocontrol isolates were significantly more effective in reducing the incidence and severity of wilt disease under greenhouse conditions compared to ACC deaminase-non-producing biocontrol isolates. The application of ACC deaminase-producing isolates resulted in the reduction of the endogenous levels of ACC, the immediate precursor of ethylene, in both roots and shoots and increased plant growth compared to isolates that did not produce the ACC deaminase. Endophytic actinomycetes were recovered from inside tomato roots at all samplings up to 12 weeks after inoculation indicating that the roots of healthy tomato may be a habitat for the endophytic actinomycetes. This study is the first record of control of a soil-borne plant pathogen by endophytic actinomycetes capable of producing ACC deaminase.

Plant growth promotion and biological control of *Sclerotinia minor*, the causal agent of lettuce basal drop, by endophytic actinomycetes under UAE tunnel-house conditions

K. EL-TARABILY (1)
(1) Department of Biology, Faculty of Science, United Arab Emirates University, Al-Ain, 17551, United Arab Emirates
Phytopathology 98:S51

Fifty-two endophytic actinomycetes were isolated from within surface-sterilized lettuce roots. Four isolates which produced cell-wall degrading enzymes (chitinase and beta-1,3-glucanase) and siderophores were examined for their ability to suppress the growth of *Sclerotinia minor*, the causal agent of basal drop of lettuce (*Lactuca sativa* L.) in the United Arab Emirates (UAE). Two of the four isolates also produced antifungal metabolite(s) that significantly reduced the growth of the pathogen *in vitro*. When the pathogen was presented as the sole carbon source, all four isolates caused plasmolysis and cell wall lysis. In addition to their antagonistic activities, the four isolates also produced a variety of plant growth regulators. *Streptomyces griseorubens* produced indole-3-acetic acid (IAA), indole-3-pyruvic acid (IPYA), isopentyl adenine (iPA), and gibberellic acid (GA3); *Actinoplanes arizonensis* produced IAA, GA3 and iPA; *S. griseinus* produced GA3, iPA and *Micromonospora chersina* produced IAA and iPA. Under tunnel-house conditions, the isolates were recovered from inside the root at all samplings, up to 8 weeks after inoculation. When applied individually or in combination to seedlings, they significantly promoted plant growth and reduced the level of disease incidence under commercial tunnel-house conditions. The combination of the four isolates resulted in significantly better disease suppression and plant growth promotion, than when the plants were exposed only to individual isolates. The endophytic actinomycetes used could replace the currently recommended fungicides for this disease in the UAE. This study indicates that there is a clear need to include endophytic actinomycetes in programs aimed at the use of microorganisms to enhance plant productivity on a field scale in nutrient impoverished sandy soils.

Identification of species of *Botryosphaeria*, *Pestalotiopsis* and *Phomopsis* in blueberry in Chile

J. G. Espinoza (1), E. X. Briceno (1), B. A. LATORRE (1)
(1) Facultad de Agronomia, Pontificia Universidad Catolica de Chile, Santiago, Chile
Phytopathology 98:S51

Blueberry (*Vaccinium* spp.) production has increased considerable in Chile, today with over 8,500 ha, across a range of diverse soil conditions and climate zones. The objective of this study was to identify the species of wood pathogens associated to stem canker and dieback commonly observed in blueberry in Chile. Symptoms consisted on reddish to dark brown necrotic lesions on twigs and stems, cankered tissues below the bark, dark brown vascular discoloration and foliar necrosis and dieback. Eventually disease plants died. Disease incidence varied from 15 to 45%. A total of 22 plantings were surveyed between 2005 and 2007. On the basis of colony characteristics, conidia morphology and molecular analysis of the ITS region of the genomic DNA, and/or beta-tubulin gene and elongation-factor-1alpha, the following fungi were identified *Botryosphaeria australis*, *B. dothidea*, *B. parva*, *B. ribis*, *Neofusicoccum corticosae*, *N. mediterraneum*, *Pestalotiopsis clavispora*, *P. neglecta*, *Truncatella angustata*, *Phomopsis viticola* and *Ph. australafricana*. These species were found alone or they were co-isolated from samples taken in the same blueberry planting. Out of 82 samples, species of *Pestalotiopsis* were the most frequently isolated fungi (60%) followed by species of *Phomopsis* (24%) and *Botryosphaeria* (16%). With the exception of *Ph. viticola* all other species were pathogenic in detached blueberry stems and in 2-yr-old blueberry plants cvs. cv. O'Neal. These pathogens were consistently isolated from inoculated plants, confirming Koch's postulates. Therefore, these results demonstrate that the stem canker and dieback of blueberry in Chile can be due to species of the genus *Botryosphaeria*, *Pestalotiospsis* and *Phomopsis*.

Watermelon vine decline in southwest Puerto Rico

C. ESTEVEZ DE JENSEN (1), J. V. Rodrigues (2), L. Polanco (1)
(1) University of Puerto Rico, Crop Protection Department, Mayagüez, Puerto Rico; (2) University of Puerto Rico, Crop Protection Department, Rio Piedras, Puerto Rico

Phytopathology 98:S52

Watermelon (*Citrillus lanatus* Thunb.) is grown under irrigation in southwest Puerto Rico. Losses due to watermelon vine decline (WVD) are greater than 50 percent during the wet and temperate months. In a randomized complete block design with four repetitions, five treatments were tested: 1) control (no spray), 2) endosulfan, *Bacillus thuringiensis* (every 2 weeks) and methomyl (6 wk), 3) manzate (1 wk), chlorotalonil (2 wk), pyraclostrobin (3 wk), kocide (3 wk), thiophanate-methyl (4 wk), and then repeat the series every following week, 4) fungicides and insecticides (same as 2 and 3) and 5) UV reflective mulch and *B. thuringiensis* (every 2 wk). Samples collected at 55 days after transplanting (DAT) tested positive for potyvirus (95 percent), ZYMV (36 percent) and 0.8 percent tested positive for CMV. Three plants enclosed with screen boxes were asymptomatic and negative for viruses. The serology test showed a potyvirus different from ZYMW and PRSV. RT-PCR tests confirmed the presence of a potyvirus. Virus symptom incidence at 45 DAT was 26 percent (control), 24 percent(fungicide), 13 percent (UV), and 1 percent (insecticide). Typical virus symptoms were leaf curling, blisters and internode necrosis. At 60 DAT wilt symptoms appeared and progressed to plant death in 3 to 5 days. Whitefly (*Bemisia tabaci*) populations at 7 and 15 DAT were higher in the control than in the insecticide treatments. At 30 DAT no differences were detected. Regardless of the treatment, only one harvest of watermelon was possible due to WVD. The insecticide and fungicide treatments yielded 12 t/acre, followed by insecticide (11 t/acre), UV plastic (7 t/acre), fungicide (5 t/acre) and control (2.5 t/acre). A program of integrated practices including resistant cultivars will be required to sustain watermelon production in Puerto Rico.

Use of protein arrays for rapid and sensitive diagnostics of grapevine diseases

A. FABRITIUS (1), L. Dubrovsky (1), L. Kumagai (1), M. Trinh (1), A. Turnquist (1), Y. Zong (2)
(1) Agri-Analysis LLC, Davis, CA; (2) Full Moon Biosystems Inc., Sunnyvale, CA

Phytopathology 98:S52

Protein arrays are solid-phase ligand-binding systems that employ immobilized proteins on solid surfaces such as glass, membranes, particles, or microtiter wells. Protein arrays are rapidly becoming established as a powerful technique not only in diagnostics but also in pharmaceutical research and development to study protein-protein interactions and to monitor protein expression profiling. Arrays allow parallel multiplex screening of thousands of interactions, protein-antibody, protein-protein, protein-ligand or enzyme-substrate screening. In the microarray or chip format, such assays can be carried out with minimum use of materials while generating large amounts of data in short time. As diagnostic devices, microarrays allow multiplexing simultaneous analyses of different samples and repeated analyses of the same sample. To utilize protein arrays in plant disease diagnostics, antibodies for various grapevine viruses including grapevine leafroll viruses (GLRaV), grapevine virus A (GVA), grapevine fanleaf virus (GFLV), grapevine fleck virus, tomato ringspot virus (ToRSV) and Xylella fastidiosa (Pierce's disease) were printed using an arraying instrument in six replicates on surfaces of standard 76 × 25 × 1 mm (3 in. × 1 in. × 1 mm) polymer coated glass slides. Sample extracts from infected and non-infected grapevines were over-laid on top of the slides, and bound antigens were detected with a pool of secondary antibodies labeled with fluorescent dyes. By coupling a microarray-ELISA format with the signal amplification of tyramide deposition, the assay sensitivity was increased to as low as pg/mL. The arrays are very stable, can be stored at 4°C for three months and one year at –20°C. In this presentation we will report results from these studies in simultaneous companion with standard ELISA and PCR assays. Protein arrays are believed to be a useful tool for rapid and sensitive diagnostics of grapevine diseases.

Selection of phage-displayed peptides that inhibit soybean rust

Z. D. FANG (2), J. E. Schoelz (2), G. Stacey (2), F. J. Schmidt (1), J. T. English (2)
(1) Division of Biochemistry, University of Missouri; (2) Division of Plant Sciences, University of Missouri

Phytopathology 98:S52

Soybean rust is a recently introduced disease that continues to threaten U.S. soybean production. With limited availability of effective disease resistance in soybean germplasm resources, soybean rust control will continue to depend mainly on chemical treatments. We are developing combinational, phage-display technologies as a potential alternative mode of rust management through deployment of defense peptides. Candidate defense peptides were recently identified through bio-panning experiments. In these experiments, we incubated germinated uredospores of *Phakopsora pachyrhizi* with a phage-display library expressing random 12-mer peptides. After three rounds of phage recovery and amplification, individual phage clones with high binding affinity were randomly selected and tested for their abilities to inhibit germlings. *In vitro* testing showed that some of the peptides effectively inhibit spore germination and rate of development. Discovered candidate defense peptides are currently being evaluated in planta for infection inhibition. Initial results indicate that peptides applied in combination with *P. pachyrhizi* uredospores inhibit or delay rust pustule formation, compared to leaf inoculation with uredospores alone.

Involvement of sensor kinase gene (*skrp 1122*) for biocontrol activity by *Pseudomonas synxantha* BG33R

G. FANG (1), V. C. Waldrop (1), W. P. Wechter (3), D. A. Kluepfel (2)
(1) Clemson University, Clemson, SC, USA; (2) USDA, ARS, CPGRU, Davis, CA, USA; (3) USDA, U.S. Vegetable Laboratory, Charleston, SC, USA

Phytopathology 98:S52

Previous research identified *Pseudomonas synxantha* BG33R as potential carrier for a nematode egg-kill factor. Further research indicated that BG33R exhibits a broad-spectrum of antagonistic activity against oomycetes, fungi, nematodes and insects. Earlier screening for negative egg-kill factor indicated the presence of a GacS sensor kinase gene regulating pleiotropic traits (*skrp1122*) was necessary for the biocontrol activity. Sequencing the *skrp1122* locus revealed a co-lineage of its putative cognate partner, a response regulator GacA homolog, and multiple genes involved in membrane transportation. Functional analysis of the *skrp1122*-negative disruption mutant revealed a pleiotropic defect for most biocontrol activities and altered colony morphology. The impaired antimicrobial activity against Magnaporthe grisea of the *skrp1122* (-) mutant was carefully examined. The mutant was no longer able to suppress vegetative growth, conidiation, and appressorium formation of the fungus, nor was it able to protect rice plants from rice blast disease. Our results indicate an essential role of the GacS/GacA two-component system in the biocontrol activity in this organism.

Biosynthesis of loline alkaloids in fungal endophytes

J. R. FAULKNER (1), M. J. Spiering (1), R. B. Grossman (1), C. L. Schardl (1)
(1) University of Kentucky, Lexington, KY, USA

Phytopathology 98:S52

Loline alkaloids are *exo*-1-aminopyrrolizidines with an oxygen bridge between carbons 2 and 7. These alkaloids are produced by fungal endophytes that live in symbiosis with cool-season grasses. Lolines are insecticidal and act as feeding deterrents; however, they have little or no anti-mammalian activity. A 9-gene cluster involved in loline-alkaloid production in *Epichloë* (holomorphic) and *Neotyphodium* (anamorphic) species has been described. *Neotyphodium uncinatum*, the only species known to produce loline alkaloids in culture, was fed isotopically labeled amino acids and chemically synthesized deuterated compounds representing putative intermediates. Subsequent GCMS analysis indicates that homoserine and proline are precursors to lolines, and identified two pathway intermediates. *N. uncinatum* has two copies of the loline gene cluster, *LOL*1 and *LOL*2, which militates against gene knockout strategies to determine *lol* gene functions. However, the *lolP*2 gene in the *LOL*2 cluster is nonfunctional, so the sole active copy, *lolP*1, was eliminated by gene replacement. This resulted in an altered loline alkaloid profile that indicated that lolP is a P450 monooxygenase required for conversion of *N*-methylloline to *N*-formylloline. Results of these studies and bioinformatic analyses of gene sequences have led us to hypothesize a pathway for loline alkaloid biosynthesis, which involves condensation of proline and homoserine as the first committed step, followed by pyrrolizidine ring formation, then ether bridge formation. Expression of *lol* gene products in yeast is underway to determine their specific roles in the pathway.

Fungal pathogens (mis-) identification: A case study with DNA barcodes on *Melampsora* rusts of white and aspen poplars

N. Feau (2), M. Allaire (2), A. VIALLE (3), P. Tanguay (2), D. L. Joly (3), P. Frey (1), R. C. Hamelin (2)
(1) Institut National de la Recherche Agronomique, Centre de Recherches de Nancy, Champenoux, France; (2) Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, Sainte-Foy, QC, Canada; (3) Universite Laval, Quebec, QC, Canada

Phytopathology 98:S52

Melampsora is one of the largest rust genus including species which occur mainly on poplars and willows but alternate on a diverse range of hosts. Wide variation and overlap in morphological characters has led to confusion in species concepts within this genus. This is particularly true in the *Melampsora* section described on *Populus*, where the number of accepted taxa ranges

between 11 and 34 according to different taxonomic schools. We applied a DNA barcode system based on Internal transcribed spacers (ITS), ribosomal large subunit sequences (28S) and mitochondrial cytochrome oxidase 1 (CO1) sequences to *Melampsora* leaf rusts from white and aspen poplars in order to evaluate species delimitation within this taxonomically difficult group. This fungal group includes the *M. populnea* complex with the highly destructive pine-shoot twist rust agent *M. pinitorqua* which alternates between pines and poplars. Species were initially defined through morphometrical data and host specificity. Morphological species were then overlaid with the molecular barcodes obtained. We conclude that DNA barcodes, specifically those defined on ITS and 28S sequences, provide a highly accurate means of identifying and resolving *Melampsora* taxa. Using our DNA barcoding approach on field and herbarium specimen collected in North America with a reference set of rust species, we highlighted mis-identification in rust species from some Canadian herbariums related to either *M. abietis-canadensis* or *M. aecidiodes* species. Finally we confirm that, although they share a telial host (*P. alba*) these species are unrelated to the rusts of the *M. populnea* complex.

Effect of thrips (Thysanoptera: Thripidae) damage in the severity of purple blotch disease of onion caused by *Alternaria* sp. under tropical conditions

M. FELICIANO (1), L. I. Rivera-Vargas (1), I. Cabrera (1)
(1) University of Puerto Rico
Phytopathology 98:S53

Recently, new species of thrips (Thysanoptera: Thripidae) affecting vegetables have been reported in Puerto Rico. Little is known about the effects of thrips populations in the severity of *Alternaria* sp. in onion (*Allium cepa* L.) under tropical conditions. To determine thrips role in the development of purple blotch disease, two field experiments were conducted in 2005 and 2006. Five different treatments were evaluated: 1) Fungicide (i.e. iprodione) and natural thrips infestations; 2) Insecticides (i.e. cypermethrin and avermectin) and *Alternaria* sp. inoculation; 3) *Alternaria* sp. inoculation and natural thrips populations; 4) Insecticides and Fungicides; and 5) Negative control. Disease severity and thrips damage were evaluated using a visual scale ranging from 1 to 5 (1 = 0% and 5 = 76–100%) at 14 and 45 days after *Alternaria* sp. inoculation (DAI). Thrips number per plant was counted and species identified. Overall, disease severity increased as thrips numbers increased. Immature thrips populations were higher during 2006 (318 thrips) compared to 2005 (170 thrips). As a result, for 2006 disease severity was estimated from 51 to 75% at 14 DAI and 75 to 100% at 45 DAI, for both treatments 3 and 5. During 2005, disease severity was estimated from 26 to 50% at 14 and 45 DAI with the same treatments. Chemical treatments significantly control thrips infestations and disease severity compared to the non-chemical treatments but no absolute control was observed. Plants in treatments 3 and 5 showed more lesions per leaf, had shorter leaves compared to plants in treatments 1, 2 and 4. *Frankliniella fusca* was the most abundant species in both experiments. *Frankliniella occidentalis*, *F. schultzei* and *Thrips tabaci* were also observed. To reduce disease impact, a suitable management of thrips populations incorporating an overall Integrated Pest Management (IPM) program is necessary.

Examination of variation among isolates of *Colletotrichum* species causing chili anthracnose worldwide

C. Feng (3), S. B. WARE (3), K. Cornelius (3), B. Gabor (1), T. L. Harp (2), J. C. Correll (3)
(1) Seminis, Woodland, CA, USA; (2) Syngenta, Vero Beach, FL, USA; (3) University of Arkansas, Fayetteville, AR 72701, USA
Phytopathology 98:S53

Colletotrichum species cause anthracnose diseases on many diverse plant hosts, including economically important cereals, vegetables, and fruits. Although anthracnose of red chili pepper fruit is well-documented, anthracnose of green chili pepper fruit has recently emerged as an important disease problem in many countries around the world. The objective of the research was to characterize the diversity of *Colletotrichum* isolates recovered from chili fruit with anthracnose symptoms from a worldwide collection as well as to compare these isolates using traditional taxonomic criteria. Isolates from Brazil, Korea, Taiwan, Thailand, and the United States were examined for DNA RFLP and sequence variation of a 900-bp intron in the glutamine synthetase (GS) gene. Isolates also were compared for mtDNA RFLPs, VCG and conidial and appressorial size and shape. The RFLP and sequence variation of the GS intron could be used to clearly demarcate *C. acutatum*, *C. gloeosporioides*, *C. capsici*, and *C. boninense*. *C. acutatum* was identified as the predominant species associated with anthracnose of green chili fruit. Sequencing of the GS intron revealed 100% identity for many isolates from Korea, Thailand, Brazil, and the United States, and none of the isolates differed by more than two bp, with the exception of one isolate from Brazil which differed by 7 bp. The majority of the isolates of *C. acutatum* examined also belonged to a single VCG, CA01 and a single mtDNA RFLP haplotype. Isolates in VCG CA01 may represent a globally distributed clone of *C. acutatum* from pepper.

Root infections of *Phytophthora ramorum* and *Phytophthora kernoviae* in UK woodlands

E. J. FICHTNER (2), D. M. Rizzo (2), S. Kirk (1), A. Whybrow (1), J. Webber (1)
(1) Forest Research, Farnham, Surrey, UK; (2) UC Davis, Davis, CA, USA
Phytopathology 98:S53

Rhododendron ponticum, an invasive weed pervading UK woodlands, supports prolific sporulation of *Phytophthora kernoviae* and *Phytophthora ramorum*. The long-term efficacy of *R. ponticum* removal from woodlands as an inoculum management strategy is unknown, in part due to lack of knowledge of pathogen persistence in roots and emerging seedlings. The potential for both pathogens to infect *R. ponticum* roots was investigated. Adventitious roots from shoot layers and associated leaf litter, as well as rhizosphere soil, and foliage were collected from infested sites in 2007. Soil, leaf litter, foliage, and surface sterilized roots were baited with rhododendron leaf disks for *Phytophthora* spp. The potential for infection of *R. ponticum* seedlings in a woodland cleared of *R. ponticum* in 2005 for management of *P. kernoviae* was similarly investigated. Emergent seedlings were excavated and their surface-sterilized roots and foliage were assessed for infection by *P. kernoviae*. Neither pathogen was routinely baited from rhizosphere soil, but both were frequently recovered from leaf litter. Both pathogens were recovered from adventitious roots, and *P. kernoviae* was recovered from roots of emergent seedlings. *P. kernoviae* was routinely recovered from roots of otherwise asymptomatic seedlings. The results suggest that the persistence of these pathogens in roots and litter should be considered when managing the diseases in infested woodlands.

hrpW* is a critical virulence factor of *Xanthomonas citri* ssp. *citri

J. F. FIGUEIREDO (2), J. B. Jones (2), J. H. Graham (3), F. F. White (1)
(1) Kansas State University, Manhattan, KS 66506; (2) University of Florida, Gainesville, FL 32611; (3) University of Florida, Lake Alfred, FL 33850
Phytopathology 98:S53

Harpin and harpin-like proteins are secreted by type III secretion system (T3SS) and, although prevalent components of the T3SS, their function in virulence is not well understood. Harpins are T3SS proteins and some such as *hrpN* in *Erwinia amylovora* are pathogenicity determinants. In *Xanthomonas citri* ssp. *citri* (Xcc), hrpW encodes a homolog of the E. amylovora HrpW protein (NCBI # AAC62314), which is secreted in a T3SS-dependent manner and consists of two domains including a harpin-like domain and pectate lyase domain. In Xcc, *hrpW* is not in close proximity of the hrp cluster, which is unique from *E. amylovora*. *hrpW* mutants of Xcc were not pathogenic in grapefruit leaves. T3SS function of the *hrpW* mutant was examined using a reporter gene, *avrGf1* that elicits HR in grapefruit. The *hrpW* mutant was unable to induce HR while the wild type carrying the gene elicited HR after 4 days. We also provide evidence that the absence of hrpW impairs the survival of the bacterium in planta. Studies to understand the complexity of the interaction of the plant pathogen are currently being investigated.

Genes of *Xanthomonas citri* ssp. *citri* involved in disease development

J. F. FIGUEIREDO (2), J. B. Jones (2), J. H. Graham (3), F. F. White (1)
(1) Kansas State University, Manhattan, KS 66506; (2) University of Florida, Gainesville, FL 32611; (3) University of Florida, Lake Alfred, FL 33850
Phytopathology 98:S53

Type III effector proteins are injected into cytoplasm by the type III secretion system (T3SS) to modulate the host cell responses to bacterial ingress. Comparatively little is known about the role that specific type III virulence factors play in citrus canker disease. Using a marker exchange mutation strategy, more than 20 *Xanthomonas citri* ssp. *citri* (Xcc) mutants were generated and tested for their contribution to disease development in grapefruit. Mutations in the T3SS regulatory and structural component genes, *hrpG* and *hrpA*, respectively, resulted in loss of pathogenicity. Mutation in hpaF, a conserved gene of the T3SS and not normally associated with severe pathogenicity defects, disrupted the ability of the bacteria to produce symptoms in young grapefruit leaves. However, mutation in *hpa1*, which in *X. oryzae* pv. *oryzae* had reduced pathogenicity, and did not impair disease development. Mutations in genes for endopeptidase, actin-ADP-ribosylating toxin domain protein, and GGDEF domain protein also resulted in loss of pathogenicity. While studying the core components of the T3SS is valuable to understanding the disease process, continued investigation of novel virulence factors will undoubtedly reveal other mechanisms which provide the bacterium with an advantage over the host.

Roelfs F2007, a new bread wheat variety with improved resistance to stripe rust and leaf rust for southern Sonora, Mexico

P. FIGUEROA-LOPEZ (3), G. Fuentes-Davila (3), R. P. Singh (1), J. Huerta-Espino (2)
(1) CIMMYT, Km 45 carret. Mexico-Veracruz, El Batan, Texcoco, Edo. de Mexico, Mexico; (2) Campo Experimental Valle de Mexico, INIFAP. Km 38.5 carret. Mexico-Texcoco, Chapingo, Edo. de Mexico, Mexico; (3) Campo Experimental Valle del Yaqui, CIRNO-INIFAP. Apdo. Postal 515. C. Norman E. Borlaug Km 12, Cd. Obregon, Sonora, Mexico
Phytopathology 98:S54

Southern Sonora is the main wheat producing area in Mexico, where 255,666 ha were planted under irrigation during the crop season 2007–2008. Although durum wheat (*Triticum turgidum*) predominates, bread wheat occupies about 20% of the area. Historically, leaf rust (*Puccinia triticina*) and Karnal bunt (*Tilletia indica*) were the most important diseases. However, since 2000, stripe rust have been increasingly important, as new populations of *Puccinia striiformis* established in the region. Tacupeto F2001 is currently the most popular bread wheat cultivar with 22,900 ha planted, but has shown moderate susceptibility to stripe rust (up to 80% severity), moderate resistance to leaf rust (up to 40% severity) and has needed fungicide control. Roelfs F2007, selected from (Tacupeto F2001*2/Kukuna) has similar agronomic characteristics, Karnal bunt resistance and grain yield potential, as compared to Tacupeto F2001, as well as improved resistance to stripe rust, leaf rust and quality characteristics. Roelfs F2007 is known to have, from its Kukuna parent, the *Lr34/Yr18* complex, which is allowing this cultivar to express an infection type of moderate resistance to stripe rust (up to 40% severity) and to leaf rust (up to 20% severity). Roelfs F2007 is intended to displace Tacupeto F2001 in the regional cultivar mosaic in the next two to three years.

Comparison of techniques used for the detection of *Plum pox virus* when using different source material and sampling time

N. FIORE (2), C. Araya (1), A. Zamorano (2), F. Gonzalez (2), R. Mora (1), I. Rosales (1)
(1) Instituto de Investigaciones Agropecuarias (INIA), Santiago de Chile, Chile; (2) Universidad de Chile, Facultad de Ciencias Agronómicas, Santiago de Chile, Chile
Phytopathology 98:S54

Plum pox potyvirus (PPV), the economically most important stone fruit virus in the world, was identified in Chile in 1992. The only PPV strain present in the country is the D type. Since 1994, stone fruit plant nurseries have been following a Mandatory Official Control of PPV to avoid the spread of the virus. This procedure is conducted by using techniques such as DASI-ELISA with universal 5B monoclonal antibody and/or RT-PCR with universal P1-P2 primer set, according to the period when the sampling is performed. Our interest was to compare these two techniques through out a year when using different infected tissues (leaves, flowers, phloem and dormant buds) in a universe of 30 infected symptomatic and non-symptomatic trees. DASI-ELISA showed low levels of virus detection no matter what month of the year or type of tissue was sampled. In this case the positives PPV trees ranged from 3.6 to 7.1%. In contrast, when using RT-PCR, the positives trees ranged from 3.6 to 89.3%. The best results were obtained when using: leaves in February, March and September; dormant buds in July and flowers and phloem in August. Although we reached higher levels of detection when using the RT-PCR, we could not reach 100% of positives plants at any of the sampling times. This may be due to the uneven distribution of the virus in the infected trees, which is particularly difficult to overcome in asymptomatic trees. The importance of these results for the control of PPV in Chile regarding the time frame of the sampling and the technique used for PPV detection will be discussed. This research was supported by project SAG C4-89-14-15.

Towards the elimination of ergot alkaloid biosynthesis genes in *Neotyphodium coenophialum*

S. FLOREA (1), C. Machado (1), D. Panaccione (2), C. Schardl (1)
(1) University of Kentucky, Department of Plant Pathology, Lexington, KY, USA; (2) West Virginia University, Division of Plant and Soil Sciences, Morgantown, WV, USA
Phytopathology 98:S54

Endophytes associated with cool season grasses elicit considerable ambivalence improving plant productivity and tolerance to biotic and abiotic stresses, but producing ergot alkaloids. Cattle and horses that graze on tall fescue pastures infested with *N. coenophialum* can suffer from fescue toxicosis, symptoms of which are similar to those caused by *C. purpurea*. *N. coenophialum* strain e19 from tall fescue cv. Kentucky 31 carries *dmaW*1 and *dmaW*2, two homologous genes that encode dimethylallyltryptophan synthase, the enzyme for the first step in ergot-alkaloid biosynthesis. The goal of this research is to have a modified strain of *N. coenophialum* that does not produce the toxic ergot alkaloids but retains all the beneficial characteristics which can be used in the field application. In our effort to disrupt both homologs and ultimately obtain marker-free mutants, we are using a marker-exchange strategy employing the Cre/loxP site-specific recombination system. Several transformation methods were used to replace *dmaW*2 with the mutant *dmaW* containing a hygromycin resistance gene (*hph*) flanked by loxP sites. Of 1522 transformants obtained and screened, three were likely *dmaW*2 disruptants because they gave no PCR product from the wild-type locus, but yielded the fragment from the disruption construct. The protoplasts of the *dmaW*2-knockouts have been transformed with pKAES175 plasmid containing *Protub2cre* and no resistance marker. The protoplasts were grown on unselective media and more than 500 putative transformants were screened for the excision of the hph gene located between the loxP sites. We identified 18 transformants that have the *hph* gene looped out. This result gives us the possibility to use the same marker for sequential knockout of *dmaW*1.

OxyR, a regulator of the hydrogen peroxide stress response in *Ralstonia solanacearum* is necessary for full virulence on tomato plants

Z. FLORES-CRUZ (1), C. Allen (1)
(1) University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 98:S54

In response to pathogen attack, plants produce an oxidative burst of reactive oxygen species (ROS) that signal defenses and may have antimicrobial effects. However, little is known of the effect of oxidative stress on pathogens. The bacterial wilt pathogen *Ralstonia solanacearum* expresses likely oxidative stress response genes during tomato pathogenesis. Analysis of the available genomes of *R. solanacearum* suggests this bacterium is exposed to oxidative stress since each encodes at least 16 predicted ROS detoxification enzymes. We hypothesize that the oxidative stress response of *R. solanacearum* contributes to pathogen survival in the host and to disease development. To test this we created an *oxyR* mutant in *R. solanacearum*; *oxyR* is a positive regulator of the hydrogen peroxide stress response. The *oxyR* mutant was catalase negative and did not grow well on plates unless catalase was added exogenously. Growth in the presence of hydrogen peroxide was significantly slower than the parental strain; however, the *oxyR* mutant grew faster than wild-type in the presence of the superoxide generator Paraquat. In addition, the *oxyR* mutant was significantly reduced in virulence on tomato plants in a naturalistic soil soak assay. These results support the hypothesis that plant ROS have direct antimicrobial effects, and indicate that *R. solanacearum* confronts a stressful oxidative environment during growth inside compatible hosts.

Reservoir hosts of *Xylella fastidiosa*, causal agent of Pierce's disease of grapevines, in North Carolina

L. E. FLOYD (1), T. B. Sutton (1)
(1) North Carolina State University, Raleigh, NC, USA
Phytopathology 98:S54

Pierce's disease (PD) of grapevines is caused by the xylem-limited bacterium *Xylella fastidiosa* (*Xf*) which is vectored by leafhopper insects. PD causes mortality in grapevines and is considered the most formidable obstacle to the production of *Vitis vinifera* in the southeastern U.S. *Xf* has an extensive host range with over 160 plant species known to harbor PD strains. Vegetation surveys were conducted in three North Carolina vineyards in the Spring and Fall of 2007 to identify plant species that occur on the vineyard floor, determine the percentage of the vineyard floor covered by each species and sample the most abundant species for *Xf*. ELISA and PCR were used to test for the presence of *Xf* in each sample. Ten of 27 plant species sampled in the Spring and 11 of 23 species sampled in the Fall tested positive for *Xf* using ELISA. *Trifolium repens*, white clover, tested positive for *Xf* in 6 of 32 samples taken in Spring, and was found in two of three vineyards. *T. repens* comprised 23 percent and 12 percent of these vineyards. In Fall surveys, *Digitaria* sp., crabgrass, was found in all vineyards sampled, and comprised 30, 16 and 10 percent of the vineyard floors. *Digitaria* sp. tested positive for *Xf* in 14 of 47 samples. Results of this research will aid growers in managing PD by identifying the most important reservoir hosts of *Xf* that need to be removed in order to reduce the inoculum of *Xf* in vineyards.

Effect of glyphosate on foliar diseases in Roundup Ready alfalfa

D. Foster-Hartnett (2), D. A. SAMAC (1)
(1) USDA ARS, St Paul, MN 55108; (2) University of Minnesota, St Paul, MN, USA
Phytopathology 98:S54

Foliar diseases are a serious problem for alfalfa management in all areas where alfalfa is grown. Defoliation due to foliar diseases varies from 3–71% depending on time of year, environmental conditions, and locale. Fungicide treatments are cost-effective in only some years and locations. Recently, glyphosate was shown to be effective in reducing rust diseases in glyphosate-resistant wheat and soybean. Glyphosate inhibits 5-enol-pyruvyl shikimate 3-phophate synthase (EPSPS), an enzyme found in plants, fungi, and bacteria. Plants engineered for glyphosate resistance with a glyphosate-insensitive

EPSPS take up and translocate the herbicide throughout the plant. In greenhouse experiments we found that application of glyphosate at the lowest recommended field application rate completely controls alfalfa rust (*Uromyces striatus*) on 90% of 4-week old seedlings when plants are inoculated with the fungus 3 days after glyphosate treatment. In addition, excellent control of rust was obtained when glyphosate was applied 4 days after rust spores, indicating that the herbicide has protective and curative activity. Protection of plants from powdery mildew (*Erysiphe pisi*) was also observed. Preliminary experiments suggest glyphosate treatment reduces seedling death due to anthracnose (*Colletotrichum trifolii*). These results indicate that glyphosate could be used to help manage foliar diseases in Roundup Ready alfalfa.

Soil application of imidacloprid and related SAR-inducing compounds produces effective and persistent control of citrus canker

M. I. FRANCIS (1), A. Redondo (1), J. K. Burns (1), J. H. Graham (1)
(1) University of Florida, Citrus Research and Education Center, Lake Alfred, FL, USA
Phytopathology 98:S55

Soil drenches of the systemic insecticide imidacloprid (Admire®) produce season-long control of citrus canker caused by *Xanthomonas citri* ssp. *citri* (Xcc). Imidacloprid (IM) is a neonicotinoid that breaks down in planta into 6 chloronicotinic acid, a closely-related compound to the systemic acquired resistance (SAR)-inducer isonicotinic acid (INA). Potted seedlings of Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*) were treated with IM, INA and the inducer acibenzolar-s-methyl (ASM) as soil drenches or as a spray of foliage (ASM) one week prior to inoculation of immature leaves with Xcc. Plants were cut back and re-inoculated four times over a 6 month period. SAR induction was confirmed by expression of the PR gene, beta-1,3 glucanase. Soil drenches of IM, INA and ASM induced a high and persistent upregulation of PR gene expression and reduced canker lesions for up to 6 months compared to less than a month for foliar ASM. Soil inducers of SAR reduced canker lesions up to 70% compared with the untreated check (UTC). Lesions were small, necrotic and flat compared to pustular lesions on UTC leaves. Populations of Xcc per leaf were reduced 1-2 log units in soil treated plants.

***In vitro* inoculation of citrus germplasm for rapid screening of resistance to citrus canker**

M. I. FRANCIS (1), J. H. Graham (1)
(1) University Florida, Citrus Research and Education Center, Lake Alfred, FL, USA
Phytopathology 98:S55

A protocol for rapid screening citrus germplasm for resistance to citrus canker (*Xanthomonas citri* ssp. *citri*, Xcc) was developed for evaluation of limited quantities of leaf material derived from somatic hybridizations, transformations and plants transiently expressing genes. Xcc inoculum densities from 10^4 to 10^8 cfu/ml were infiltrated into surface disinfested, immature leaves. Inoculated leaves were placed on the surface of 0.5% water agar plates and incubated at 28°C under a 12 hour light photoperiod. At higher bacteria inoculum densities, resistant cultivars of Kumquat (*Fortunella* spp.), Meiwa and Nagami, developed a hypersensitive-like reaction within the infiltrated area delimited by the main leaf veins within a period of 72 h. No symptoms or a few small necrotic spots developed at the lower inoculum densities. Susceptible *Citrus* spp., rough lemon (*C. jambhiri*), Volkamer lemon (*C. volkameriana*) and grapefruit (*C. paradisi*) infiltrated with the same inoculum densities produced no tissue alterations 72 hrs after inoculation. For susceptible cultivars, symptoms required 168 h or longer to develop water-soaking and tissue hypertrophy typical of citrus canker lesions in compatible hosts.

Fine-scale kinetic changes in *Arabidopsis thaliana* physiology during the hypersensitive response suggest a two-layered defense strategy that prevents bacterial invasion and halts infection

B. C. FREEMAN (1), G. A. Beattie (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 98:S55

The hypersensitive response (HR) is a plant defense reaction characterized by rapid programmed cell death localized to the site of infection. Our previous studies suggested that the *Arabidopsis thaliana* response to an avirulent derivative of *Pseudomonas syringae* pv. tomato DC3000 involves both rapid water loss and virtually complete restriction of vascular water flow into the infection site. The purpose of this study was to characterize the kinetics and mechanisms of HR-associated drying at the infection site in *A. thaliana* leaves, including plant responses to bacteria introduced via vacuum-infiltration or immersion. Following vacuum-infiltration, changes in leaf weight indicated a rapid and significant drying within only 8 h. Moreover, significant vascular restriction occurred within only 3 h and was highly correlated with an increase in the number of dead cells, suggesting the possibility that the two events are related. We applied an LI-6400 photosynthesis and gas exchange system to monitor the fine-scale kinetic changes in *A. thaliana* physiology in attached, intact leaves following infection. Stomatal conductance and transpiration exhibited large and rapid reductions over the first 6 h following vacuum-infiltration, accompanied by a sharp decrease in photosynthetic activity between 4 and 6 hpi. In contrast, leaves inoculated by immersion exhibited rapid stomatal closure but delayed onset of vascular restriction indicating that the two events are separable. We propose that *A. thaliana* utilizes a two-layered defense strategy during the hypersensitive response: a strong and sustained closure of stomata, which restricts further bacterial entry, and vascular restriction in the infection site, which promotes localized tissue drying and thus inhibits growth of the invading pathogen.

Detection of multiple strains of the aster yellows phytoplasma in Wisconsin carrot fields

K. E. FROST (2), C. L. Groves (1), R. L. Groves (1)
(1) Department of Entomology, Russell Laboratories, University of Wisconsin, Madison, WI 53706; (2) Department of Plant Pathology, Russell Laboratories, University of Wisconsin, Madison, WI 53706
Phytopathology 98:S55

Each year, Wisconsin carrot fields are threatened by the occurrence of aster yellows phytoplasma (AYp), which is obligately transmitted by the aster leafhopper (*Macrosteles quadrilineatus* Forbes). AYp acquisition and transmission and AYp latent period within host plant and leafhopper are important factors that influence epidemic development in a given year. There is evidence that these factors are influenced by pathogen strain variability. However, there have been no studies that have characterized the genetic diversity of AYp in Wisconsin susceptible crops or the genetic relationships associated with ability of an AYp-variant to cause disease on carrot. We have initiated studies to determine if the AYp strains present in Wisconsin leafhoppers and carrot fields and are similar to pathotypes previously described in Ohio. To date, 80% of the symptomatic plants collected from 8 commercial carrot fields in 2007 have tested positive for the presence of AYp by a PCR assay. Two AYp strains previously characterized in Ohio have been detected in carrot and multiple strains are present in leafhoppers. Other etiological agents, such as, beet leafhopper-transmitted virescence agent and *Spiroplasma citri*, have not been detected in the symptomatic carrot plants that tested negative for AYp or in leafhoppers. Results from these studies have already provided new information about the predominant AYp strains present in Wisconsin and will provide a better understanding of the importance of pathogen variability in relation to the distribution and spread of aster yellows in susceptible crops.

Soybean plants with reduced levels of oleic acid show increased resistance to multiple pathogens

D. FU (1), S. Ghabrial (1), A. Kachroo (1)
(1) University of Kentucky
Phytopathology 98:S55

Stearoyl-acyl carrier protein-desaturases (SACPDs) catalyze an essential step in fatty acid biosynthesis leading to the formation of oleic acid (18:1). Arabidopsis mutants (ssi2) with reduced SACPD activity accumulate salicylic acid (SA) and exhibit enhanced resistance to multiple pathogens. We used a bean pod mottle virus (BPMV)-based vector to silence SACPDs in soybean. Our studies show that reduced levels of 18:1 also alter defense signaling in soybean, even though soybean and Arabidopsis differ significantly in their lipid biosynthetic pathways. Soybean plants silenced for SACPDs develop spontaneous cell death lesions, increase SA accumulation, and constitutively overexpress pathogenesis-related genes. Silencing SACPDs also induced increased expression of resistance (R)-like genes and enhanced resistance to bacterial and oomycete pathogens. Similar phenotypes could be induced in healthy soybean plants via exogenous application of glycerol. Our results suggest that soybean and Arabidopsis respond similarly to 18:1-derived cues by inducing a novel broad-spectrum resistance-conferring pathway. In addition to SACPDs, we have used the BPMV-based vector to examine the defense-related functionalities of several soybean sequences. Virus-induced silencing of endogenous sequences in soybean has allowed us to identify components essential for R gene-mediated resistance to viral and bacterial pathogens. Identification of such components could aid in the development of novel means for engineering disease resistance in soybean.

Development of a plant expression vector based on cucumber mosaic virus with truncated 3a protein

M. FUJIKI (1), J. Kaczmarczyk (1), S. Rabindran (1), V. Yusibov (1)
(1) Fraunhofer USA Center for Molecular Biotechnology, Newark, DE, USA
Phytopathology 98:S55

We have developed a Cucumber mosaic virus (CMV)-based expression vector for the production of heterologous proteins in plants. Cell-to-cell movement of CMV is normally dependent on the presence of coat protein (CP). Previous

studies have shown that deletion of 33 amino acids from the carboxy-terminus of the 3a movement protein facilitates cell-to-cell movement that is independent of CP. The CMV-based expression vector that we have designed utilizes this truncated 3a protein, and thus allows the expression of target genes from the strong CP subgenomic promoter without the need for providing CP in trans for cell-to-cell spread. In combination with Agrobacterium-mediated delivery, accumulation of green fluorescent protein (GFP) at approximately 300 mg/kg was achieved in Nicotiana benthamiana plants. Human growth hormone accumulated at ~170 mg/kg.

Effect of sorghum seedlings of different genotypes, and previous crop, on soil microorganism populations

D. L. FUNNELL (1), J. F. Pedersen (1), D. B. Marx (2)
(1) USDA-ARS, Lincoln, NE, USA; (2) University of Nebraska, Lincoln, NE, USA
Phytopathology 98:S56

Hypotheses that sorghum seedlings of different genotypes will differentially modify soil microorganisms and will affect subsequently planted wheat seedlings were tested. Wheat cultivar Lewjain, and sorghum genotypes Redlan and RTx433 were planted into soils previously planted with wheat or sorghum. Fungal and fluorescent *Pseudomonas* spp. numbers (cfu) were significantly affected by plant species, sorghum genotype and previous crop. Soils planted with RTx433 or Lewjain had significantly greater numbers of fungal cfu than soils planted with Redlan. When Lewjain seedlings were grown in soil previously planted with RTx433, there were greater numbers of fungal cfu than when Lewjain was planted into Redlan soil. Wheat planted into wheat soil resulted in significantly fewer numbers of fluorescent *Pseudomonas* spp. than when planted into sorghum soil. Fluorescent pseudomonads were assessed for the presence of a gene for 2,4-diacetylphloroglucinol production (*Phl*). Percentage of isolates with *Phl* declined for most treatments but increased when Redlan was planted in Redlan soil. When rifampicin-marked *Pseudomonas fluorescens* isolates were applied to soils, sorghum seedlings sustained rhizosphere and soil populations similar to those of wheat. There may be differences between sorghum genotypes regarding associations with soil microorganisms, suggesting that sorghum genotypes may differentially affect numbers of soil microorganisms in cropping systems.

Validated Ca. Liberibacter asiaticus genomic DNA contigs assembled using a metagenomics approach

D. W. GABRIEL (1), S. L. Reddy (1)
(1) University of Florida, Gainesville, FL 32611, USA
Phytopathology 98:S56

Citrus Huanglongbin (HLB), also known as citrus “greening” is a lethal disease of citrus that is now widespread in Florida. Based primarily on 16S rDNA sequence analysis, HLB is associated with at least three different species of *Candidatus* Liberibacter. None of the candidate species have been cultured; consequently Koch’s postulates have not been completed to confirm Liberibacter as the sole causal agent. The titer of these phloem limited bacteria is so low that the genome size has not been estimated and < 25 kb of nonredundant genomic DNA has been sequenced, primarily by PCR methods. Nearly all PCR tests to confirm HLB are based on two genomic regions, and phylogenetic information relevant to strain identity has not been assessed. Using dodder transmission, we have continuously transmitted HLB from a single infected Florida citrus tree to citrus and periwinkle for two years; by PCR, this HLB sample is associated with Ca. L. asiaticus (Las). We developed a DNA extraction protocol that enriched for Las and greatly reduced chloroplast and mitochondrial DNA contamination (as determined by PCR) and used multiple displacement amplification (MDA) to obtain sufficient DNA for shotgun library sequencing. To date, 6,000 sequencing reactions from this library have resulted in the identification of >40 kb of new Las DNA sequence in 10 new contigs that have been validated by PCR primers designed from the new contigs. One of the contigs, currently 7.2 kb in size, completely encompasses and extends the existing 988 bp singlet for DNA polymerase from Las (GenBank M94320). Twelve new primer sets have been developed and validated against Florida Las samples; some primers revealed potential phylogenetic differences between the Florida and one Brazilian Las samples tested.

Age demographics, hiring trends, and graduation rates in plant pathology in the United States

D. GADOURY (1), A. Lichens-Park (6), J. Andrews (9), J. MacDonald (7), K. Baumgartner (6), M. Kennelly (3), T. Burr (1), A. Talley (5), S. Savary (2), H. Scherm (8), G. Wang (4)
(1) Cornell University; (2) INRA, Bordeaux; (3) Kansas State University; (4) Ohio State University; (5) Syngenta; (6) USDA; (7) University of California, Davis; (8) University of Georgia; (9) University of Wisconsin
Phytopathology 98:S56

We examined the status of plant pathology departments and age demographics of the profession. Seven of eight large departments have lost from 17 to 40% of their faculty positions since 1987, and several smaller graduate programs in plant pathology (e.g., in several northeastern states) have all but disappeared. A census of plant pathology faculty at U.S. universities revealed a median age of 52, with marked compression around the median. This predominant cohort was directly attributable to hiring during the period from 1966 to 1985, after which hiring rates dropped by nearly 50% for 20 years. Institutional expansion was followed by reduction, depressing numbers in all cohorts younger than the median age. Accelerated retirements are projected to begin in 2009 and the rate will steadily increase before peaking in 2016 at approximately five times the 2008 rate. At the same time, the annual number of PhDs awarded in plant pathology dropped by 15% during 2001 to 2005 from the previous 35-year period. Although focused on university faculty, the demographic trends are also broadly applicable to government service and the private sector. While our findings indicate improved job prospects for Plant Pathology graduates in the near term, the profession will simultaneously lose those best suited to mentor the broadly-trained professionals that are presently in demand. The aforementioned trends would be best addressed by strategic planning of national scope.

Genetic characterization of predominantly nivalenol-producing populations belonging to the *Fusarium graminearum* species complex from the Southern U.S.

L. R. GALE (1), S. A. Harrison (2), E. A. Milus (3), K. O’Donnell (5), T. J. Ward (5), H. Kistler (4)
(1) Dept. Plant Pathology, University of Minnesota, St. Paul, MN, USA; (2) Dept. of Agronomy, Louisiana State University, Baton Rouge, LA, USA; (3) Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR, USA; (4) USDA, ARS, Cereal Disease Laboratory, St. Paul, MN, USA; (5) USDA, ARS, National Center for Agricultural Utilization Research Laboratory, Peoria, IL, USA
Phytopathology 98:S56

Surveys into the genetic diversity of the *Fusarium graminearum* species complex, the main causal agent of *Fusarium* head blight in small grains have revealed that isolates in Louisiana are genetically distinct from isolates in the midwestern U.S. While most isolates from the Midwest belong to a genetically homogeneous deoxynivalenol-producing population (MW15ADON), isolates belonging to the F. *graminearum* species complex in Louisiana are predominantly nivalenol (NIV) producers. The objectives of this study were to characterize the fungal diversity in the southern U.S. and to evaluate its genetic relationship with the MW15ADON population. Isolates were analyzed from 12 southern and midwestern states (AL, AR, IL, IN, KS, LA, MO, MS, NC, NE, OH, TX). Trichothecene type (3ADON, 15ADON, or NIV) was established for all examined isolates using a multiplex PCR-test; their chemotype was validated for a subset of isolates in greenhouse experiments. Ten polymorphic molecular markers, i.e. PCR-RFLPs were developed in highly polymorphic regions of the genome, and a multilocus genotype based on these markers established for each of 635 isolates. Clone-corrected data was further analyzed with STRUCTURE 2.2. Isolates with a nivalenol trichothecene type were predominant in Louisiana (80% of strains), but did not belong to a single population. Most of these nivalenol-producing isolates could be distinguished into a Southern Louisiana population and a previously described Gulf Coast population, and both belonging to *F. graminearum sensu stricto*. Nivalenol-producing members of *F. graminearum sensu stricto* were also identified from Arkansas, North Carolina and Missouri. In addition, based on a Luminex test, nivalenol-producing members of *Fusarium asiaticum* were found to be present in Louisiana.

Investigating sources of genetic variability of *Phytophthora nicotianae*, the causal agent of black shank of tobacco in NC

C. A. GALLUP (1), K. L. Ivors (1), H. Shew (1)
(1) North Carolina State University, Plant Pathology, Raleigh, NC, USA
Phytopathology 98:S56

The black shank pathogen occurs in all tobacco-producing areas of North Carolina. The state has experienced rapid race shifts since the wide-spread deployment of single gene resistance. Previous investigations suggested that this pathogen is highly variable, potentially contributing to the rapid rate of race shifts in the population. It is unknown whether the high level of population diversity is due to asexual recombination alone, or if sexual sporulation contributes to the epidemiology of the disease. Current investigations conducted in the laboratory, greenhouse, and field indicate high levels of variability contributed by asexual sporulation. Also, a multiyear state-wide survey of NC revealed that both mating types of *Phytophthora nicotianae* occur throughout the state. Both mating types were present in at least 28% of the fields surveyed, and sexual compatibility was identified between isolates within two of the fields surveyed. Characterizing the level of genetic variability arising from asexual and sexual recombination will help elucidate how variability is generated and how it influences pathogen phenotypes, including races. This information can be used by breeders and pathologists

interested in the development and deployment of different types of resistance genes and their short and long term viability for disease management.

Fusarium and _Rhizoctonia_ species associated with root rots of dry beans in North Dakota and Minnesota

A. GAMBHIR (1), R. S. Lamppa (1), J. B. Rasmussen (1), R. S. Goswami (1)
(1) North Dakota State University, Dept. of Plant Pathology, Fargo, ND 58105, USA
Phytopathology 98:S57

Root rot of dry beans (*Phaseolus vulgaris*) is caused by a complex of several organisms, including *Fusarium solani* f. sp. *phaseoli* and *Rhizoctonia solani*, which are thought to be most common in the production areas of North Dakota and Minnesota. In order to assess the prevalence of these and other root rotting pathogens, a survey of five dry bean growing counties in North Dakota and Minnesota was conducted during the summer of 2007. Plants of three different market classes (kidney, pinto and navy beans) expressing root rot symptoms were collected from 40 commercial fields. Potential pathogens were isolated from roots in the laboratory. Initial identification of fungal species was conducted according to morphological characteristics. Other experiments compared a region of the TEF-1 gene for *Fusarium* species and the ITS region of *Rhizoctonia* spp. with publicly available sequences from members of these genera. Several Fusarium species, including, *F. oxysporum*, *F. solani*, *F. acuminatum* and *F. redolens* were isolated from these roots. *Rhizoctonia solani* was generally found in association with *Fusarium* sp. in most fields. To our knowledge, this is the first association of *F. acuminatum* and *F. redolens* with root rot of dry beans in this region. Experiments are underway to test these species for pathogenicity on dry bean roots.

Characterization of mango malformation disease and the interaction between the pathogen *Fusarium mangiferae* and the mango bud mite *Aceria mangiferae*

E. GAMLIEL-ATINSKY (2), E. Palevsky (1), A. Sztejnberg (3), D. Shtienberg (2), M. Maymon (2), S. Freeman (2)
(1) Department of Entomology, Newe-Ya'ar Research Center, ARO, Ramat Yishay 30095, Israel; (2) Department of Plant Pathology, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan 50250, Israel; (3) Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Israel
Phytopathology 98:S57

Mango malformation, caused by *Fusarium mangiferae*, is one of the most destructive diseases of this crop. Very little is known about the disease epidemiology and the possible association between the mango bud mite *Aceria mangiferae* and the fungal pathogen. Research objectives were to assess primary inoculum in the orchard, study patterns of conidial dispersal, locate the infection sites and the means of reaching it, and, determine the role of the bud mite in epidemiology of disease. Malformed inflorescences were assessed in a diseased orchard and significantly more conidia/g infected panicles were found in May and June than in April during 2006 and 2007, corresponding with higher numbers of malformed inflorescences per tree and a peak in dispersal of inoculum. No windborne bud mites bearing conidia were found in mite traps. Inoculation of leaves, branches, and buds resulted in colonization only in buds, suggesting that buds are the exclusive infection sites. Following exposure of the mite to a green fluorescent protein-marked isolate, conidia were observed clinging to its body. In inoculation experiments on leaves of mango seedlings, conidia were found inside buds only when both mites and conidia were co-inoculated on the plant, demonstrating that mites vectored conidia into apical buds. Dry malformed panicles that were placed on apical buds of potted plants were found to be infective. A significantly higher disease incidence was recorded in buds inoculated with conidia in the presence of mites than with conidia alone, demonstrating the significance of the mite in the fungal infection process. This study sheds new light on the epidemiology of the disease and the role of *A. mangiferae*, particularly on the mode of vectoring conidia of *F. mangiferae* and assisting fungal penetration. We believe that these novel findings are important steps towards developing an improved strategy for management of mango malformation disease.

Molecular variability of *Grapevine leafroll-associated virus-1* in the Pacific Northwest vineyards

K. Gandhi (1), T. A. Mekuria (1), R. R. Martin (2), R. A. NAIDU (1)
(1) Department of Plant Pathology, Washington State University, Irrigated Agric. Res. Extn. Ctr, Prosser, WA 99350; (2) USDA-ARS Horticulture Crops Research Lab, 3420 NW Orchard Ave., Corvallis, OR 97330
Phytopathology 98:S57

Grapevine leafroll disease (GLD) is the most economically important viral disease in the Pacific Northwest. *Grapevine leafroll-associated virus-1* (GLRaV-1) is one of six GLRaVs documented so far in the region. A study was undertaken to determine molecular variability of GLRaV-1 collected from two wine grape and two ornamental grape cultivars. A 398 nucleotide (nt) fragment specific to the coat protein duplicate 2 (CPd2) was amplified from eight GLRaV-1 isolates by reverse transcription-polymerase chain reaction (RT-PCR). In addition, a 633 nt fragment of the open reading frame 9 (ORF 9), located towards the 3′ end of the virus genome, was amplified by RT-PCR from the two ornamental grape cultivars. The CPd2- and ORF 9-specific amplicons were cloned and sequenced, and the sequences compared among themselves and with corresponding sequence of GLRaV-1 isolate from Australia (GenBank accession number AF195822). The CPd2 sequences showed nucleotide sequence identity between 93 and 99% and amino acid sequence identity between 89 and 100% among the eight GLRaV-1 isolates. However, these isolates showed 85–88% nucleotide sequence identity and 80–84% amino acid sequence identity with the corresponding CPd2 sequence of an Australian isolate. ORF 9 sequences from the two ornamental grapes showed 98% identity among themselves at both nucleotide and amino acid level and 88% identity with the Australian isolate at both nucleotide and amino acid level. These results suggest genetic diversity among GLRaV-1 isolates from the Pacific Northwest region and indicate their differences with GLRaV-1 isolate from Australia. Additional data on sequence variation in other genomic regions of GLRaV-1 will be presented.

Maize lipoxygenase ZmLOX3-mediated pathway suppresses seed colonization, production of spores and mycotoxins by *Aspergilli* spp.

X. GAO (1), T. Isakeit (1), M. Brodhagen (3), N. P. Keller (2), M. V. Kolomiets (1)
(1) Texas A&M University, College Station, TX, USA; (2) University of Wisconsin-Madison, Madison, WI, USA; (3) Western Washington University, Bellingham, WA, USA
Phytopathology 98:S57

Both plant and fungal lipoxygenases catalyze formation of a large family of poly-unsaturated fatty acid oxide compounds, collectively called oxylipins. Previous data showed that fungal oxylipins are required for fungal development and secondary metabolism. It is hypothesized that plant host-derived oxylipins may mimic or interfere with fungal oxylipins to regulate these processes in fungi. To address genetically this question, we used *Mutator*-insertional *lox3* mutants and tested their interactions with the mycotoxigenic seed-infecting fungus *Aspergillus flavus* and its non-pathogenic relative *Aspergillus nidulans*. The results showed that *lox3* mutant is more susceptible to both *A. flavus* and *A. nidulans*, which displayed increased sporulation and mycotoxin production, compared to wild type, suggesting that ZmLOX3-mediated pathway inhibits pathogenic development of this fungus. Moreover, interaction between both *lox3* and defined *A. nidulans* oxylipin mutants indicated existence of complex oxylipin-mediated signaling between the host and the pathogen that governs the outcomes of their interactions.

Comparative structural genomics of disease resistant wild potato species comprising the tertiary genepool of cultivated potato

L. GAO (1), M. Iorizzo (2), H. S. Mann (1), D. Carputo (3), J. M. Bradeen (1)
(1) University of Minnesota, Department of Plant Pathology, St. Paul, MN, USA; (2) University of Minnesota, St. Paul, MN USA and University of Naples "Federico II", Portici, Italy; (3) University of Naples "Federico II", Portici, Italy
Phytopathology 98:S57

The potato tertiary genepool consists of wild species that are sexually incompatible with cultivated potato. These species are rich sources of resistance genes to biotic and abiotic stresses, yet remain largely untapped. Here we report the development of genome-wide linkage maps for two potato tertiary genepool species, *Solanum bulbocastanum* and *S. commersonii* using Diversity Array Technology (DArT). These species represent phylogenetically distinct series within the potato tertiary genepool. We used mapping populations consisting of 92 F_1 progeny. The DArT array built for this study has 16,500 features, of which 9,500 were generated from tertiary genepool genomes. Preliminary data on polymorphism between the mapping parents suggest resulting linkage maps will be comprised of 750 and 1,000 markers for *S. bulbocastanum* and *S. commersonii*, respectively. Resulting maps can be further augmented with other marker sources and will be the first medium density genome-wide linkage maps for these species. The DArT platform will be tested for other tertiary genepool species, enabling cross-species comparisons. With ongoing genome sequencing projects in tomato and cultivated potato, sequencing of DArT markers mapped in wild potato will allow comparison of genome-wide structure throughout the genus *Solanum.*

Host specificity and population structure of *Aspergillus* section *Flavi* in sugarcane production areas in the Rio Grande Valley of Texas

N. P. GARBER (1), P. J. Cotty (2)
(1) The University of Arizona, Tucson, AZ, USA; (2) USDA-ARS, The University of Arizona, Tucson, AZ, USA
Phytopathology 98:S57

Aspergillus section *Flavi* communities associated with sugarcane production in the Rio Grande Valley of South Texas are dominated by one non-native B

and G aflatoxin-producing morphotype, called FP-1. FP-1 isolates are transported globally in association with the billets used to propagate sugarcane. FP-1 isolates have been associated with economically important raw sugar contamination events in Japan. The distributions of the FP-1 morphotype in soil and among and within sugarcane plants in the Rio Grande Valley were investigated. FP-1 isolates from Texas were characterized morphologically, biochemically, and by phylogenetic and vegetative compatibility analyses. In the absence of sugarcane FP-1 in soils rapidly declined below detectable levels within 5 years of cane termination regardless of the rotation crop. This is the first example of host specificity for an aflatoxin-producing *Aspergillus*. The results describe introduction of a toxin-producing *Aspergillus* into a non-native agricultural production area, a phenomenon that may have occurred elsewhere cryptically.

Microarray analysis of deoxynivalenol-induced gene expression in susceptible (cv. Morex) barley

S. A. GARDINER (1), H. Jia (1), J. Boddu (1), G. J. Muehlbauer (1)
(1) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S58

Fusarium head blight (FHB) is a devastating fungal disease of barley (*Hordeum vulgare* L.). FHB is primarily caused by the fungal pathogen *Fusarium graminearum*. The yield and quality of infected grain is severely reduced because of blighted kernels and the presence of deoxynivalenol (DON), a trichothecene mycotoxin produced by the fungus. Our objective was to examine the barley host response after DON application. A susceptible barley genotype inoculated with DON was shown to convert DON to DON-3-glucoside in planta. In a subsequent experiment, a susceptible barley genotype (cv. Morex) was inoculated with the equivalent of 2.0 ng DON per floret or mock-inoculated with water. Microarray analysis was conducted with the Barley1GeneChip® to examine gene expression at 1, 12, 24, 48 hours after inoculation. A total of 255 individual transcripts were significantly upregulated, with a fold change $\geq$2.0 between DON and water treatment. Eleven transcripts were significantly downregulated (fold change $\leq$ 0.5 between DON and water treatment). Transcripts were annotated and grouped into functional classes, which included: defense, metabolism, signal transduction, regulatory, and trichothecene detoxification. Comparative analysis with Fusarium-barley studies show that many of these genes may be DON-specific. Gene expression of a subset of genes from the microarray experiment was evaluated in two barley near-isogenic pairs that carry resistant and susceptible alleles at two FHB resistant QTL.

Influence of pH on pathogen inhibition by streptomycetes

A. A. GARZA (3), R. Trevino (1), C. R. Little (2), A. Davelos Baines (1)
(1) Department of Biology, The University of Texas – Pan American, Edinburg, TX; (2) Department of Plant Pathology, Kansas State University, Manhattan, KS; (3) Department of Plant Pathology, University of Minnesota, St. Paul, MN
Phytopathology 98:S58

A variety of soilborne fungal pathogens infect cucurbits in the Lower Rio Grande Valley (LRGV) of Texas. Streptomycetes are well known for their antibiotic producing capabilities and for their ability to suppress the growth of various microorganisms. For this reason, they are promising biocontrol candidates. Twelve streptomycetes with varying inhibitory activities were tested under a range of pH levels to determine the effect on inhibitory activity towards fungal pathogens. Standardized amounts of streptomycete isolates were inoculated *in vitro* and tested against each of five fungal pathogens including *Fusarium oxysporum* f. sp. *lycopersici*, *Didymella bryoniae*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Aspergillus niger*. Starch casein agar (SCA) was amended with lactic acid to adjust pH levels to 6.0, 6.5, 7.0, 7.5 and 8.0. The maximum zone of inhibition varied with each fungal pathogen and streptomycete isolate combination; however, for the majority of interactions, acidic conditions produced significantly smaller zones of inhibition. There was no significant difference for *A. niger*, regardless of pH level, while most isolates tested against *M. phaseolina* and *D. bryoniae* exhibited significantly larger zones at basic pH levels. Very few isolates were able to inhibit *R. solani* and *F. oxysporum*. Isolates that were able to inhibit *R. solani* were not significantly different over the pH range tested. Isolates that inhibited *F. oxysporum* exhibited significantly larger zones at more basic pH levels. These results illustrate the potential influence of abiotic factors on successful biological control.

Low-doses of fungicides have a stimulatory effect on *Pythium* spp. *in vitro* and *in planta*

C. D. GARZON (1), G. W. Moorman (3), J. M. Yánez (3), J. E. Molineros (2), R. C. Leonard (3), M. Jimenez-Gasco (3)
(1) Dept. Biology, The College of Wooster, Wooster, OH; (2) Dept. Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; (3) Dept. Plant Pathology, Pennsylvania State University, University Park, PA
Phytopathology 98:S58

The observation of fungi and oomycetes growing on solid media amended with fungicides faster than on non-amended media has been regularly disregarded as experimental error. However, complains of growers that disease incidence occasionally increases after application of fungicides suggests that there may be something behind the experimental observations other than simple errors. To determine if plant pathogenic oomycetes of the genus *Pythium* were stimulated by low concentrations of fungicides *in vitro*, isolates of two *Pythium* species (*P. aphanidermatum* and *P. cryptoirregulare*), one sensitive to mefenoxam and one resistance to the fungicide, were grown on cornmeal agar (CMA) amended with 11 different concentrations of mefenoxam (1×10^2 ul/ml to 1×10^{-20} ul/ml) or CMA alone (positive control). Twenty-four hours after inoculation the radial growth of each isolate was measured. Treatments where the microorganism grew faster in presence of the fungicide than in the positive control were considered to have a stimulant effect. The strain of *Pythium* that displayed the most significant stimulatory response to low-doses of mefenoxam (P18, *P. aphanidermatum*, resistant) was selected for additional testing. In order to determine if concentrations of fungicides in fertilizer solutions equivalent to those tested *in vitro* would stimulate the pathogen and result in higher levels of disease *in planta* (damping-off), geranium seedlings were inoculated with P18. Disease progress was evaluated every 24 hrs for 7 days. Our results indicate that exposure to low doses of mefenoxam can stimulate resistant strains of *Pythium*, inducing a statistically significant increase of *in vitro* growth, and a higher incidence of damping-off of seedlings.

Post inoculation moisture and deoxynivalenol production by *Fusarium graminearum* in wheat

P. GAUTAM (1), R. Dill-Macky (1)
(1) Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S58

A field experiment (split-split-plot, 5 reps) was conducted in 2007 to examine the effect of moisture on the production and accumulation of deoxynivalenol (DON) in *Fusarium*-infected wheat. Main plots were duration of mist-irrigation [14, 21, 28 and 35 d after inoculation (DAI)]; sub-plots, wheat cultivar (n = 3); and sub-sub-plots *F. graminearum* (Fg) isolate (n = 5) differing in aggressiveness and DON production capacity. The cultivars Alsen (moderately resistant, Sumai-3 derived), 2375 (moderately susceptible) and Wheaton (susceptible) were inoculated at anthesis (1×10^6 macroconidia mL^{-1}). Severity was assessed 21 DAI (20 heads/plot) and heads harvested (10/plot) at 0, 7, 11, 14, 21, 28, 41 DAI for DON analysis. Visually scabby kernels (VSK) and DON were determined on grain harvested at maturity. Severity, VSK and DON, across all isolates, were significantly higher in Wheaton. Severity and VSK were significantly lower in the treatments receiving the least amount of mist-irrigation (14 DAI) suggesting that extended moisture promotes disease development. DON was significantly lower in the 35 d misting treatment. DON in head samples was reduced with increased durations of irrigation, but was only significantly lower in grain from the 35 d misting treatment. The reduction of DON was larger in Wheaton than other cultivars. Our results suggest that DON may be reduced by late-season moisture despite increased grain colonization. Leaching may explain the reduction of DON observed with an increased misting duration, and differences in tissue morphology and metabolism may determine the rate of leaching from specific tissues.

Disease management in production of certified seed potatoes by organic practices

R. K. GENGER (1), D. I. Rouse (1), A. O. Charkowski (1)
(1) Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 98:S58

Many important potato diseases can be transmitted in seed potatoes. Certified seed potatoes, which are inspected to ensure that pathogen levels are below a specified threshold, provide effective control of most tuber-borne diseases in potato. In Wisconsin, the aphid- and tuber-borne disease Potato Virus Y (PVY) is the most significant disease in seed potato production. Organic growers, who must attempt to source organically-grown seed, face a shortage of organically-grown certified seed potatoes in the Midwest. To test the feasibility of certified seed potato production under organic management, we conducted trials on six Wisconsin organic farms. Trials included two strategies for control of aphid-transmitted PVY: wheat borders and weekly mineral oil sprays. At a field site in northern Wisconsin, PVY incidence in harvested tubers from all plots was below the certification threshold, and PVY was not detected in 44 of 48 plots tested. At a field site in south-west Wisconsin, PVY incidence exceeded the certification threshold for 3 of 44 plots, and PVY was not detected in 34 of 44 plots tested. Harvested tubers were screened for a range of other pathogens, visually and using an oligonucleotide macroarray. Surface defects due to common scab and silver scurf were sufficient to affect certification in some cases. As disease pressure

and aphid incidence vary yearly, trials will be repeated to determine long-term feasibility of seed potato production on Wisconsin organic farms.

Comparison of ELISA, PCR, and a new TaqMan real-time PCR test for *Clavibacter michiganense* subsp. *sepedonicus*
D. J. GERHARDT (1), A. Phibbs (2), A. O. Charkowski (1)
(1) University of Wisconsin-Madison, Madison, WI, USA; (2) Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, WI, USA
Phytopathology 98:S59

Bacterial ring rot, caused by the pathogen Clavibacter michiganense subsp. sepedonicus (CMS), is a serious pathogen of potatoes and a zero tolerance pathogen in seed potato production. Current detection methods include ELISA and PCR, tests which have been shown to be variable. In the fall of 2006 several non-seed potato farms in central Wisconsin had outbreaks of ring rot and we randomly sampled tubers from these fields. A sample (1 cm × 1 cm) from the stem end of the potato was placed in sterile water and placed on a shaker overnight. The suspension was divided for ELISA and PCR testing. To develop a TaqMan real-time PCR assay sequences specific to CMS were used to construct two sets of primers and probes. A total of 184 samples were tested and 37.5% were positive with ELISA. Results from the PCR tests using three primer sets were: 51.6%, 50.0%, and 55.9% positive, respectively. The next step in the project will be to test all samples with our TaqMan real-time assay and determine if there is a correlation between tests.

Dose response of soilborne pathogens to acrolein
J. S. GERIK (1), D. Wang (1)
(1) USDA-ARS, Parlier, CA, USA
Phytopathology 98:S59

Growers of cut flowers, strawberries and other high value crops in California have routinely used pre-plant methyl bromide and chloropicrin soil fumigation to control soilborne pathogens. Because of the ban on methyl bromide production and import, alternative treatments are required. One product which has been proposed as a methyl bromide replacement is acrolein (2-propenal). Laboratory test were conducted to determine the effective dose of acrolein to control various soilborne plant pathogens. Acrolein was applied with water to columns of soil to simulate an application made by drip irrigation. Twelve concentrations of acrolein were applied to represent rates of 0 – 5000 kg•ha^{-1}. The soil was infested with either *Agrobacterium tumefaciens*, *Fusarium oxysporum*, *Pythium paroecandrum*, or *Verticillium dahliae*. The acrolein mixture was applied to the top of the column and allowed to drain through the soil. The tops of the columns were sealed after application. Following treatment, populations of the pathogens were determined by dilution plating on selective media. Logistic dose-response models were used to estimate the effective concentration to reduce pathogen viability. *A. tumefaciens* and *P. paroecandrum* were the most sensitive to acrolein being controlled at rates of 1000 kg•ha-1, while *F. oxysporum* and *V. dahliae* required greater rates for control. Acrolein is currently registered as an aquatic herbicide, but is not registered as pre-plant soil treatment.

Identification of the avirlulence gene of Leptosphaeria maculans corresponding to the resistance gene LepR1 in Brassica napus through sequence related amplified polymorphic (SRAP) markers
K. Ghanbarnia (2), D. G. FERNANDO (2), G. Li (2), R. Rimmer (1)
(1) Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2; (2) Dept. of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
Phytopathology 98:S59

Blackleg is the most economically devastating disease of canola/rapeseed worldwide. Efforts are made to understand the host-pathogen interaction by studying the interaction between resistance genes of the host and the avirulence genes of the pathogen in order to stabilize resistance in pathogen prevalent areas. Previous studies showed that the double haploid line, ddm-12-6S-1 of canola, possess a novel resistance gene LepR1 against PGT (99-43 & 99-56) and PG2 (WA-74) isolates. However, this line is susceptible to PG2 isolate 87-41. It is thought that the avirulence of isolates WA-74, 99-43 and 99-56 on the line ddm-12-6-1 is governed by the Avr/LepR1 and the virulence of isolate 87-41 is governed by avr1LepR1. Isolates 87-41 and 99-56 were selected as virulent and avirulent isolates based on phenotypic reactions caused by the isolates on the line and characterization of opposite mating types. They were crossed and the progenies segregated based on the phenotypic reaction on line ddm-12-6S-1. Sequence related amplified polymorphic (SRAP) markers and bulked segregant analysis was used to produce polymorphism between avirulent and virulrnt parents and their progeny. Two markers were identified with genetic distances of 6.4 and 7.1 cM to Avr1LepR1 and a genetic map was constructed. This is the first report of and naming of the avirulence gene as Avr1LepR1 gene of L. maculans corresponding to the LepR1 gene in B. napus.

Introduction of the mycorrhizal fungus *Sebacina vermifera* into elite switchgrass (*Panicum virgatum* L.) cultivars for potential enhancement of biomass and productivity
S. R. GHIMIRE (1), K. D. Craven (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, USA
Phytopathology 98:S59

Experiments were conducted to evaluate compatibility between switchgrass (*Panicum virgatum* L.) cultivars and the root-infecting mycorrhizal fungus, *Sebacina vermifera*. Two elite switchgrass cultivars were inoculated with six strains of *S. vermifera* using three different techniques: transplanting of rooted nodal explants of switchgrass into soil amended with fungal mycelium; planting of explants shoots on rooting medium colonized by the fungus; and dipping of roots of 7 to 10 days old seedlings into a fungal mycelium suspension. Inoculated nodal explants or seedlings were transplanted into sterile soil and maintained in the glasshouse for three weeks before examination for the presence of the fungus. The presence of *Sebacina* was evaluated using three different detection techniques: polymerase chain reaction using *Sebacina* specific primers; grow out tests; and microscopic examination of stained root tissues. Root-dipping into a mycelial suspension was the technique showing the greatest promise, and we detected colonization of two fungal strains on switchgrass genotype NF/GA 993 and barley variety Golden Promise. Further studies on these associations including the stability of root colonization, as well as subsequent effects on plant establishment, biotic/abiotic stress tolerance and biomass production, using all six strains of *S. vermifera* and additional switchgrass genotypes is underway.

Association of *Seuratia millardetii* (Myriangiales) with a false sooty mold disorder of *Camellia* species
I. M. Gillis (1), D. A. GLAWE (2)
(1) University of Washington, Seattle, WA, USA; (2) Washington State Univ./Univ. Washington, Seattle, WA, USA
Phytopathology 98:S59

A sooty mold-like disorder of *Camellia* species is common in the Pacific Northwest. To identify the causal agent, samples for study were collected from the *Camellia* collection of the Washington Park Arboretum, University of Washington. The fungus on affected leaves was *Seuratia millardetii* (anamorph: *Atichia glomerulosa*), known previously from conifer species in the region. No fungi referable to Capnodiales were found. Colonies ranged from 0.1 mm to several mm across. Colonies were cushion-shaped or lobed, consisting of a peridium of melanized cells containing globose hyaline cells in a gelatinous matrix; hyphae were lacking. Sporioles, chirospores, and phialospores typical of *A. glomerulosa* were observed. The teleomorph included bitunicate asci and ascospores typical of *S. millardetii*. Observation with SEM confirmed that colonies grew superficially on leaves without penetrating them. On malt extract agar colonies resembled those on host leaves and produced sporioles and chirospores, but grew indeterminately to become several cm across. The fungus was observed on several named cultivars of *C. japonica*, *C. sasanqua*, and *C.* x *williamsii*. This appears to be the first report of *S. millardetii* on *Camellia* spp., known previously from a wide range of angiosperms and conifers. As in previous studies, no associated insects were found, suggesting the need to reinvestigate the effectiveness of attempts to control the disorder by eliminating honeydew-producing insects.

Towards undercovering the secretion mechanism of effector proteins during biotrophic invasion by the blast fungus *Magnaporthe oryzae*
M. C. GIRALDO (2), G. Mosquera (1), B. Valent (2)
(1) Bioag Sci & Pest Mgmt, Colorado State University; (2) Kansas State University, Manhattan, KS 66506
Phytopathology 98:S59

The hemibiotrophic rice blast fungus *Magnaporthe oryzae* sequentially invades living rice cells using specialized biotrophic invasive hyphae (IH). The IH are tightly enclosed in a plant membrane, the Extra-Invasive Hyphal Membrane or EIHM, as they grow inside rice cells. To accomplish its biotrophic invasion, the fungus must secrete proteins (effectors) across the EIHM inside the rice cells to turn down plant defense responses and to control plant processes and metabolism. Previous studies in the rice blast system have focused on the putative effector proteins encoded by avirulence (*AVR*) genes *AVR-Pita* and *PWL2*. Analysis of chimeric proteins produced by fusing AVR-Pita and PWL2 to the green fluorescent protein showed that they were secreted by IH and accumulated in novel structures, Blast Interfacial Complexes (BICs), located between the IH cell wall and the EIHM. We extend this analysis by examining secretion patterns for additional putative effectors; novel in planta-specific secreted proteins identified by microarray analysis. For four putative effectors, we fused enhanced yellow fluorescent protein (EYFP) at the C-terminus of the entire protein coding sequence and expressed them using the native promoter. We produced transformed fungal strains and assayed them for in planta protein secretion using the rice leaf

sheath assay. Each of these four genes were specifically expressed and secreted during biotrophic invasion of rice cells. They exhibit different patterns of secretion ranging from the BIC secretion pattern previously associated with avirulence proteins to intense uniform distribution within the EIHM around the entire IH. Challenges remain in demonstrating the delivery of fungal proteins across the EIHM into the rice cytoplasm. Ultimately, identification and characterization of rice blast effectors and understanding how they are delivered to the host cytoplasm will represent a major advance in molecular plant-pathogen interactions.

Fusarium verticillioides genes necessary for biotransformation of maize allelopathic compounds

A. E. GLENN (1), C. W. Bacon (1)
(1) USDA, ARS, Russell Research Center, Toxicology & Mycotoxin Research Unit, Athens, GA, USA
Phytopathology 98:S60

Corn produces the cyclic hydroxamic acids DIMBOA and DIBOA, which naturally transform into the more stable benzoxazolinones MBOA and BOA, respectively. These compounds are implicated in allelopathic weed suppression, insect feeding deterrence, and microbial disease resistance. *Fusarium verticillioides*, the most common fungal pathogen associated with corn, has the physiological capacity to detoxify MBOA and BOA. Biotransformation of BOA is suggested to involve hydrolysis (encoded by the *FDB1* locus) to produce 2-aminophenol (2-AP), which is subsequently acylated (encoded by the *FDB2* locus) to produce *N*-(2-hydroxyphenyl)malonamic acid (HPMA). Fungal growth is inhibited on BOA-amended medium if either locus is mutated. An *fdb2* mutant can produce low levels of an acetylated 2-AP branch metabolite, *N*-(2-hydroxyphenyl)acetamide (HPAA). Suppression subtractive hybridization was used to identify genes up-regulated in response to BOA, and two gene clusters were identified that functionally correspond to the *FDB1* and *FDB2* loci. Genes at both loci are being evaluated. At the *FDB2* locus a putative *N*-acetyltransferase (NAT) was of particular interest due to the postulated role of *FDB2* in acylation of 2-AP. This gene was subcloned from an identified cosmid that complemented an *fdb2* mutant. The subcloned gene also complemented an *fdb2* mutation. Deletion of the gene eliminated the ability of *F. verticillioides* to metabolize BOA, and the mutants did not grow. We therefore functionally associate *FDB2* as the gene encoding this putative NAT activity. The branch metabolite HPAA was produced at low concentrations in Delta*fdb2* mutants suggesting acetylation of the intermediate 2-AP occurred independently from the putative *N*-acyltransferase activity of FDB2, which is proposed to involve malonylation of 2-AP. Thus, we have provided further evidence for the genetics and biochemical nature of benzoxazolinone biotransformation that may enhance the ecological fitness of *F. verticillioides* in the cornfield environment.

Taegro: A biofungicide with broad spectrum of activity towards soilborne or foliar fungal and bacterial pathogens

S. S. GNANAMANICKAM (1), S. Inman (1), L. West (1), S. Semones (1)
(1) Novozymes Biologicals, Salem, VA 24153
Phytopathology 98:S60

Taegro is an EPA-registered biofungicide whose active ingredient is 5.0×10^{10} cfu/g of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24. It can be applied as soil drench or foliar spray at the rate of 2.5 to 3.5 oz per 100 gallons of water/A. Laboratory profiling of its bioefficacy in the laboratory has revealed that it has a wide spectrum of biological activity towards both fungal and bacterial pathogens. This includes the fungal pathogens, *Rhizoctonia*, *Fusarium*, *Phytophthora* (including mefenoxam-resistant field strains of *P. erythroseptica*) and *Venturia inaequalis* and strains of bacterial pathogens, *Xanthomonas campestris*, *Pseudomonas syringae* pathovars, *Ralstonia solanacearum*, and *Erwinia amylovora*. In greenhouse trials, we observed more than 75% reduction of bacterial wilt in tomato when Taegro was incorporated into the potting mix at the labeled rate of 1.23 g/gallon mix. Our laboratory and greenhouse observation are supported by crop data from field trials. In earlier field trials conducted during 1995–1998 treatment of tomatoes led to delay in Fusarium wilt development and also to 10-to-20% yield increases. In potatoes and ornamentals, FZB24 treatments led to consistent increase of tuber yield, marketable plants as well as number of flowers. From recent field trials conducted in different CA locations during 2007, significant disease reductions due to Taegro treatments were recorded for the suppression of (i) bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (greater than that was afforded by kocide 2000), (ii) downy mildew of organic head lettuce caused by *Bremia lactucae* when compared to chemical standards, kaligreen and other conventional chemistry combinations, and (iii) bottom rot of lettuce caused by *Rhizoctonia solani* over the chemical standard botran 75-W. These results suggest that soil drench and foliar applications of Taegro show no phytotoxicity and can be reliably used to control fungal and bacterial pathogens.

Quantification of _Tilletia indica_ teliospores in sori of commercially harvested wheat grains

B. J. GOATES (1)
(1) USDA-ARS, Aberdeen, ID
Phytopathology 98:S60

Karnal bunt caused by *Tilletia indica* is a minor disease of wheat that has caused considerable international quarantine concerns. Knowledge of the number of teliospores in sori of diseased grains is needed to provide information for the development of pest risk assessments. Eight to fourteen diseased kernels were randomly selected from each of nine diverse commercially harvested wheat samples collected during three seasons. Diseased kernels were soaked individually in 0.5% Tween for 1-2 d and then spores were carefully dissected from kernels and suspended in Tween for 1-2 d. The suspensions were vigorously vortexed for 1 minute and three, 10 µl aliquots were removed from 10 to 40 ml of total suspension for spore counts. Counts from each aliquot were similar. Mean spore counts per kernel among samples ranged from 1.2×10^4 to 3.8×10^4. Spore counts per kernel among all kernels examined ranged from 1.0×10^3 to 9.3×10^4; mean 2.3×10^4. Six kernels with intact point infections selected from the commercial grain samples had from 1.9×10^4 to 8.9×10^4 spores; mean 4.7×10^4. Spore counts from four artificially inoculated, extremely infected hand-threshed kernels with intact sori ranged from 2.6×10^5 to 5.0×10^5; mean 3.8×10^5. Such fragile kernels are normally not found intact in commercial grain. This information enables more precise estimations of teliospore numbers in infected grains which will improve the accuracy of pest risk assessments.

Pathogenic characterization of strains of the B and C group of _Xanthomonas axonopodis_ in citrus

A. M. Gochez (1), M. A. RYBAK (1), B. I. Canteros (1)
(1) INTA-BID 1728/ OC-AR PICTO 12956, EEA INTA, Bella Vista, Corrientes, Argentina
Phytopathology 98:S60

Strains of the B and C group of *Xanthomonas axonopodis* pathogenic to citrus are generally cited as members of one pathovar, *X. a.* pv. *aurantifolii*. We characterized the pathogenicity of both groups of strains. Strains of the B and C groups isolated in Argentina in 1990 (B, from lemon) and received from Brazil (C, from Key lime) were infiltrated in the mesophyll of young citrus seedlings in a growth chamber. Inocula of 5×10^3 to 5×10^5 cells per mililiter were used to infiltrate several citrus varieties, Key lime (*Citrus aurantifolia* Christm.), grapefruit (*C. paradisi* Macf), tangerine (*C. reticulata* Blanco), lemon (*C. limon* (L.) Burm.), and orange (*C. sinensis* (L.) Osb.). The B strains caused canker lesions in all citrus inoculated, the most susceptible being Key lime (3.93 to 76.25 lesions/cm^2) followed by lemon (0.3 to 2.01), tangerine (0.27 to 3.89), grapefruit (0.12 to 2.55), and orange (0.11 to 2.89). The C strains only produced canker symptoms in Key lime (1.29 a 21.46 lesions/cm^2) and did not caused any canker lesions in any other citrus type inoculated.

Window of opportunity for root infection leading to foliar symptoms of soybean sudden death syndrome

C. GONGORA-CANUL (1), L. Leandro (1)
(1) Iowa State University
Phytopathology 98:S60

Sudden death syndrome (SDS) is an economically important soybean disease caused by F. virguliforme. Foliar symptoms typically develop after flowering in field conditions, but can sometimes appear on young plants. Fungal colonization of the root vascular system is essential for expression of foliar symptoms since these are caused by a toxin translocated from the roots to the leaves. Our objective was to define the window of opportunity for effective root infection resulting in the development of SDS foliar symptoms. Soybean seeds were planted staggered over a five week period to obtain plants of different ages (1 day, 4 days, and 1, 2, 3, 4, and 6 weeks old) at inoculation. Plants were inoculated by drenching a spore suspension of 6×10^3 conidia/ml into each pot and incubated for 7 days at 17°C, followed by 31 days at 24°C. Root rot and foliar severity were assessed at 18 and 38 days after inoculation (DAI). Root rot developed on plants inoculated at all ages, but plants inoculated 1-day after planting had the highest ($P < 0.01$) root rot severity, 76.8% at 18 DAI and 90% at 38 DAI respectively, compared to those inoculated at all other ages. Root rot severity did not differ among plants inoculated 1 to 5 weeks after planting and ranged between 12–32%. Foliar symptoms only developed on plants inoculated 1-day after planting, but not on any other age. Foliar severity on these plants was 21% at 18 DAI and 80% at 38 DAI. Frequency of root colonization by *F. virguliforme* was consistent with root rot severity. This study reveals that soybean roots are susceptible for infection at all ages but that root infection at the seedling stage is critical for foliar symptom development. The effectiveness of management practices aimed at protecting seedling roots at the stage should be investigated.

Evaluation of *Agrobacterium tumefaciens* genetic diversity in CA walnut growing regions and resistance to the biocontrol agent, *Agrobacterium rhizogenes* K84

E. T. GONZALEZ (1), M. Maccree (1), M. Zaid (1), D. A. Kluepfel (1)
(1) USDA, ARS, Crops Pathology/Genetics Research Unit, Davis, CA, USA
Phytopathology 98:S61

Crown gall of walnut (*Juglans* sp.), caused by the bacterium *Agrobacterium tumefaciens*, is of great concern to the California walnut growers and nurseries due to significant yield and tree losses. To determine the genetic diversity of *A. tumefaciens* throughout the California Central Valley, we collected isolates from ten counties, which encompass the CA walnut growing regions. A total of 340 *A. tumefaciens* biovar 1 isolates were isolated from both soil and gall tissue using the *Agrobacterium* semi-selective medium 1A amended with 80 ppm potassium tellurite. A PCR-based approach was used to detect the presence of the Ti plasmid, the virulence determinant of *A. tumefaciens*. One-hundred ninety isolates were Ti-plasmid positive or shown to be virulent in bioassays. To assess genetic diversity, BOX PCR profiles were generated for the confirmed virulent isolates and analyzed using GelCompar II. Several distinct genotypes clustered as a function of geography. Due to the level of genotypic diversity observed among the *A. tumefaciens* isolates we hypothesized that control of crown gall by the biocontrol agent *A. rhizogenes* K84 would be variable as well. To this end, we tested the ability of the biocontrol agent *Agrobacterium rhizogenes* K84 to inhibit the growth of a subset of virulent *A. tumefaciens* biovar 1 isolates displaying distinct genetic fingerprints. Analysis of the genetic heterogeneity of California *A. tumefaciens* isolates will facilitate the design of effective crown gall management strategies, including judicious application of *A. rhizogenes* K84 depending on the *A. tumefaciens* genotype present.

Ancient isolation and independent evolution of the three clonal lineages of the sudden oak death pathogen *Phytophthora ramorum*

E. M. GOSS (1), N. J. Grunwald (1)
(1) USDA ARS
Phytopathology 98:S61

Sudden oak death, an emerging disease caused by the exotic pathogen *P. ramorum*, is responsible for extensive mortality of oaks and tanoaks in Northern California as well as economic losses to U.S. and European nurseries due to its infection of common ornamental plants. In its introduced range, *P. ramorum* occurs as three distinct clonal lineages. The two common lineages are opposite mating types, but oospores are not readily produced in culture and they have not been observed in the field. We inferred the evolutionary history of *P. ramorum* from DNA sequence variation at five nuclear loci using coalescent-based approaches. We found that the lineages have been diverged for at least 11% of their history, an evolutionarily significant amount of time roughly estimated to be on the order of 165,000 to 500,000 years. There was also strong evidence for historical recombination between the lineages, indicating that the ancestors of the *P. ramorum* lineages were members of a sexually reproducing population. Due to this recombination, the ages of the lineages varied within and between loci, but analyses suggested that the European lineage may be older than the North American lineages. The divergence of the three clonal lineages of *P. ramorum* supports a scenario in which the three lineages originated from different geographic locations that were sufficiently isolated from each other to allow independent evolution prior to introduction to North America and Europe. It is thus likely that the emergence of *P. ramoru*m in North America and Europe was the result of three independent migration events.

Silencing of defense-related genes reveals different mechanisms leading to race-specific resistance to Phytophthora in soybean

M. Y. Graham (3), M. R. Sinden (3), R. Huge (3), S. Subramanian (1), O. Yu (1), S. St. Martin (2), T. L. GRAHAM (3)
(1) Donald Danforth Plant Science Center, St. Louis, MO, USA; (2) Horticulture and Crop Sciences, Ohio State University, Columbus, OH, USA; (3) Plant Pathology, Ohio State University, Columbus, OH, USA
Phytopathology 98:S61

Agrobacterium rhizogenes mediated RNAi gene silencing has been used to determine the roles of several defense-related genes in a series of Williams isolines carrying various Rps genes for race specific resistance to *Phytophthora sojae*. The Rps lines examined included those carrying Rps 1, 1b, 1c, 1k, 2, 3, 3b, 3c, 4, 5 and 7. The genes silenced included isoflavone synthase (IFS) and chalcone reductase (CHR) for isoflavone biosynthesis, PR-1a, the glucan elicitor releasing endoglucanase (PR-2), a unique multidomain metallothionein gene (MMT), two soybean Npr homologs and an isoflavone specific beta-glucosidase (ISBG). Phenotypes were examined in transformed hairy roots, and included resistance or susceptibility to infection by *P. sojae* and various cell death and redox responses to infection or treatment with the cell wall glucan elicitor (WGE) from *P. sojae*. An interesting new finding was that while the isoflavones were essential to HR cell death in lines carrying Rps 2 or alleles at the Rps 1 locus, cell death in lines carrying Rps 7 or alleles at Rps 3 was isoflavone-independent. This suggests that different classes of Rps genes may modulate different cell death pathways. An additional finding is that silencing the Npr homologs led to enhanced general (partial, basal) resistance. This unexpected finding is under further investigation.

Epidemiology of *Phytophthora capsici* in water

L. L. GRANKE (1), M. Hausbeck (1)
(1) Michigan State University, Plant Pathology
Phytopathology 98:S61

Phytophthora capsici has been detected in surface water used for irrigation in Michigan, but the epidemiology of *P. capsici* in water has not been elucidated. Controlled laboratory studies were undertaken to determine the effects of water temperature (1.5 ± 0.9, 12.4 ± 0.6, 19.4 ± 0.8, 22.4 ± 0.8, and 31.6 ± 0.7°C), zoospore concentration (100, 1,000, 5,000, 10,000, 20,000, and 40,000 zoospores/ml), and zoospore suspension age on *Phytophthora capsici* infection of cucumber fruit. For each temperature/concentration combination, six unwounded pickling cucumbers were added to 3 liters of zoospore suspension, removed 24 hours later, rinsed with distilled water, incubated in a moist chamber, and cucumber tissue was excised and plated onto field isolation media. Six unwounded pickling cucumbers were added to 3 liters of zoospore suspension immediately, 1 day, 3 days, or 5 days after zoospore suspension preparation to measure zoospore longevity at 19.4 ± 0.8°C. Cucumbers were removed 24 hours later and treated as described above. Three independent experiments were conducted for each temperature, concentration, and age tested. All experiments contained a negative control (0 zoospores/ml, 19.4 ± 0.8°C). No control fruit were infected. Fruit were not infected at 1.5 ± 0.9°C, but a high percentage of infection occurred at all other temperatures tested. The percent of fruit infected at concentrations greater than or equal to 1,000 zoospores/ml was significantly higher than the control. Concentrations greater than or equal to 5,000 zoospores/ml caused a significantly higher percentage of infection than 100 zoospores/ml. The ability of zoospores to infect cucumbers was significantly reduced by day 5, but some infection was still observed. Understanding the epidemiology of *P. capsici* in irrigation water sources is essential for designing effective management strategies.

Emerging diversity in Potato virus Y poses new challenges for the U.S. potato industry

S. GRAY (4), A. Karasev (5), J. Lorenzen (2), J. Whitworth (3), P. Nolte (5), K. Perry (1)
(1) Cornell University; (2) IITA, Uganda; (3) USDA, ARS, Aberden, ID; (4) USDA, ARS, Ithaca, NY 14853; (5) University of Idaho
Phytopathology 98:S61

Until recently tobacco and potato tuber necrotic strains of Potato virus Y (PVY) were considered to be absent from the U.S., and seed certification programs were able to limit PVY incidence in seed potatoes. PVY has become more problematic in recent years and beginning in 2002, necrotic strains of PVY isolated from potato were reported from several growing regions. A three year survey of PVY strains infecting the U.S. seed potato crop was conducted by sampling tubers from most late generation seed potato fields in all production areas in 16 states. PVY isolates were characterized using serology, plant bioassays, and nucleic acid-based diagnostics. Over 200,000 tubers were tested using ELISA; nearly 9100 tested positive for PVY, and to date over 2300 isolates have been characterized using all the above mentioned diagnostics. Twelve unique phenotypic groups of PVY were identified, 5 of which were found in significant numbers. Additionally, sequence information has identified over 30 different genotypes, although most have not been linked to specific phenotypes. Interestingly recombination between necrotic and non-necrotic strains is responsible for much of the genetic diversity, with 7 unique recombinant genotypes identified to date. Another significant finding is the widespread distribution of a non-necrotic strain that has a single amino acid change in the coat protein that is recognized by a necrotic 'strain-specific' monoclonal antibody. Isolates in strain groups other than PVY^{NTN} have been observed to induce potato tuber necrotic ringspot disease, and a range of tuber symptom types have been observed. The recent emergence of genetic diversity within PVY poses a threat to potato production and requires immediate changes in PVY disease management strategies.

Genome-wide analysis of carbohydrate-active enzyme coding genes in *Phytophthora infestans*: The glycoside hydrolase gene family

J. J. GRIFFITH (1), R. W. Jones (2), M. D. Ospina-Giraldo (1)
(1) Lafayette College, Easton, PA, USA; (2) USDA/ARS, Beltsville, MD, USA
Phytopathology 98:S61

Phytophthora infestans is a plant pathogen responsible for the late-blight disease in potato and tomato crops. The infection mechanism of this oomycete may rely on cell wall degrading enzymes (CWDE) that specifically target the carbohydrates of the plant cell wall. Among the CWDE enzymes secreted by plant pathogens are glycoside hydrolases (GH), which catalyze the hydrolysis

of the glycosidic bonds that form cellulose. *Phytophthora infestans* is believed to secrete these enzymes to degrade cellulose into smaller, less complex sugars, allowing for infection through the plant cell wall. Thus far, only GH family 5 and family 12 genes have been identified in *P. infestans*. Using BLAST techniques, a wide scale analysis of the *P. infestans* genome was conducted to identify putative genes coding for other GH families, and a total of 281 genes belonging to 34 families were identified. Amplification of genes belonging to families 7 and 18 from 10 different isolates of *P. infestans* was attempted and 90% of the targeted glycoside hydrolase genes were successfully amplified. Six of the ten isolates contained all six glycoside hydrolase genes, while all but one included the majority of the genes. US-940-SO1 was the only isolate to clearly lack glycoside hydrolase genes of families 7 and 18. The presence or absence of specific glycoside hydrolase genes may play a significant role in the pathogenicity of *P. infestans*.

Phytophthora erythroseptica, isolate sensitivity to metalaxyl and disease control in potato in New York and Pennsylvania

H. M. GRIFFITHS (1), T. A. Zitter (1), K. L. Deahl (3), D. E. Halseth (2)
(1) Dept. Plant Pathology and Plant Microbe-Biology, Cornell University, Ithaca, NY 14853, USA; (2) Dept. of Horticulture, Cornell University, Ithaca, NY 14853, USA; (3) Vegetable Laboratory, BARC-West, Beltsville, MD 20705, USA
Phytopathology 98:S62

Pink rot in potato caused by *Phytophthora erythroseptica* (*P. erythroseptica*), is becoming an increasing problem for growers in New York (NY) and Pennsylvania (PA). At the end of the 2007 season, symptoms characteristic of pink rot were identified on tubers at seven farms in six counties in NY and PA. Isolation of *P. erythroseptica* was attempted and, at least nine isolates were positively confirmed by PCR. Products such as Ridomil, with the active ingredient metalaxyl, have been used routinely to control *P. erythroseptica*, but, in many locations in North America resistance has been reported. The NY and PA isolates were tested for sensitivity to metalaxyl. Two isolates were highly resistant, three moderately resistant and four sensitive. The four sensitive isolates were obtained from organic farms or fields that had not recently received a product containing metalaxyl. As the ability to control *P. erythroseptica* in potato in NY and PA using products containing metalaxyl appears to be limited, there is an urgent need for different control measures. We tested potato cultivars suitable for the region for their response to the pathogen by inoculating tubers and a range in cultivar susceptibility was detected. These cultivars along with the labeled chemicals Ranman and Phostrol will be further evaluated in field conditions where pink rot has been a problem.

Evaluation of North American potato cultivars for their resistance to potato black dot, _Colletotrichum coccodes_

E. GRIMME (1), J. R. Meyer (1), O. T. Neher (1), B. J. Jacobsen (1)
(1) Montana State University, Bozeman, MT, USA
Phytopathology 98:S62

Potato black dot, caused by the fungus *Colletotrichum coccodes* (Wallr.) Hughes is a pathogen associated with potato early dying and has been reported worldwide. The disease is characterized by the development of small black sclerotia on roots, stems, stolons, and tubers of senescent and dead potato plants. The importance of the disease has recently increased and economic losses of up to 30% have been reported. Infection of plants may be associated with soil-, tuber-, or air-borne inoculum. The purpose of this research was to evaluate North American potato cultivars for resistance to *C. coccodes* under greenhouse and field conditions. Tissue culture derived plantlets from the Montana Potato Laboratory (Bozeman, MT) of 43 commercial cultivars were artificially inoculated with *C. coccodes* at time of planting. Cultivar resistance was evaluated by monitoring stunting of plants and development of foliar symptoms like chlorosis and necrosis during the growing period. Fresh and dry weights of foliage and colony forming units (cfu) of *C. coccodes* in the lower 10 cm of stem above the soil line were determined. *C. coccodes* infection resulted in significant growth reductions ($P < 0.05$) in greenhouse and field trials. The cultivars Highland, Atlantic, Cal White, Frontier, Gem Russet, GemStar Russet, Red Lasoda, Russet, Sangre, and Viking were the most resistant cultivars in the greenhouse trials. Kennebec was the most susceptible cultivar tested with *C. coccodes* infection resulting in a 53.6% reduction in dry weight.

Comparing _Leifsonia xyli_ subsp. _xyli_ vascular colonization to yield loss for ranking susceptibility of sugarcane to ratoon stunting disease

M. P. GRISHAM (1)
(1) USDA, ARS, Sugarcane Research Unit, Houma, LA, USA
Phytopathology 98:S62

Leifsonia xyli subsp. *xyli* is a xylem-limited bacterium that causes ratoon stunting disease of sugarcane. The disease produces no characteristic external visual symptoms with the primary affect a mild to severe reduction in growth. Susceptibility of 19 commercial cultivars was determined based on the percentage of vascular bundles in the stalk colonization by the bacterium and on yield loss observed in field experiments comparing infected plants and uninfected plants. Cane and sucrose yields were determined in plant-cane, first-ratoon, and second-ratoon crops of each experiment, and the percent colonized vascular bundles was determined from 12 randomly collected stalks from each plot of the experiments using tissue-blot immunoassay. The percent colonized vascular bundles ranged from 4 to 75% among the 19 cultivars; however, mean percent did not differ among the three crops within a cultivar. Mean percent sucrose per hectare loss ranged from 0 to 28%. Mean percent colonized vascular bundles was not correlated with mean sugar yield loss indicating different mechanisms of resistance to infection and yield loss. Although no yield loss was detected in five cultivars with low to moderate percent colonized vascular bundles (7 to 35%), seed cane free of *X. xyli* subsp. *xyli* infection should be planted to avoid spread of the pathogen to cultivars susceptible to yield loss.

Defense peptides derived from combinatorial libraries as a novel means of protection against Fusarium head blight

N. W. GROSS (2), Z. D. Fang (2), F. J. Schmidt (1), J. T. English (2)
(1) Division of Biochemistry, University of Missouri; (2) Division of Plant Science, University of Missouri
Phytopathology 98:S62

Head blight is a fungal disease of wheat and barley that can cause significant economic loss. Several species of *Fusarium* are able to induce head blight, but in North America the most important is *F. graminearum* (teleomorph *Gibberella zeae*). In the spring, flowers become colonized by the fungus, resulting in infected grain that may contain damaging levels of mycotoxins. Head blight is commonly managed with fungicides. There is also an assortment of wheat and barley varieties with a range of disease resistance, but none of these offer complete protection. We are developing defense peptides that may provide a useful management option. To identify candidate peptides, we first combined germinated *F. graminearum* macroconidia (germlings) with phage-displayed libraries of highly diverse, random 8-mer peptides. We subsequently tested peptides with high germling-binding affinity for inhibitory effects. Initial *in vitro* tests have shown that affinity-selected peptides are able to delay macroconium germination and reduce germ tube elongation rates. In addition, some peptides disrupt normal, apical-cell germ tube emergence patterns. The most effective peptides will next be tested for protective abilities by *in planta* assays. At completion of these phenotype screens, we will display the best candidate peptides on recently developed scaffold proteins for delivery into plants.

Impact of Potato virus Y on long term storage of potato

R. L. GROVES (2), A. O. Charkowski (2), A. J. Bussan (2), S. M. Gray (1)
(1) Cornell University; (2) University of Wisconsin
Phytopathology 98:S62

In recent years, Potato virus Y has reemerged as a serious disease problem in many potato production areas in the northern United States and eastern Canada. In Wisconsin, two widely grown cultivars, Russet Norkotah and Silverton, express mild or no symptoms when infected with PVY. The lack of symptoms often associated with these cultivars and novel, recombinant strains of this virus prevents accurate field identification and rouging of infected plants resulting in higher levels of virus inoculum and greater disease pressure. We have initiated field studies to investigate the efficiency of tuber infection in plants inoculated with different strains of PVY at different plant developmental stages, and to document in storage quality losses associated with each PVY strain. This area of investigation seems extremely important as there have been no studies to quantify or define the consequences of long-term storage of tubers infected with PVY. To date, replicated field experiments conducted in 2007 have resulted in some overall differences in tuber yield and quality estimates among the varieties tested. Differences in percent solids, specific gravity, marketable tuber weight, marketable tuber number, and non-marketable tubers were detected in selected cultivars. This project has already generated new information regarding the relative responses of selected potato varieties to infection by the novel, recombinant PVY strains in Wisconsin while also providing practical guidance in documenting the impact of virus infection on long-term tuber storage.

Virtual lesions caused by copper-based fungicides impair photosynthesis in tart cherry

B. R. GRUBER (2), E. L. Kruger (1), P. S. McManus (2)
(1) Department of Forest and Wildlife Ecology, University of Wisconsin - Madison, Madison, WI, USA; (2) Department of Plant Pathology, University of Wisconsin - Madison, Madison, WI, USA
Phytopathology 98:S62

Leaf spot of tart cherry, caused by the fungus *Blumeriella jaapii*, presents chlorosis and necrosis. Copper-based fungicides may provide growers with a management option because fungal resistance is expected to be low. However,

foliar lesions are often associated with the use of copper. In 2007, virtual lesion size was measured, allowing us to determine if leaf spot symptoms or copper associated leaf discoloration had an effect on the remaining green leaf tissue. Net CO_2 assimilation and stomatal conductance were measured over the 2007 season in tart cherry sun and shade leaves that had been sprayed with either synthetic fungicides or copper-based fungicides, or not sprayed. Fifteen days after the first spray, significant ($P < 0.03$) virtual lesions were detected in copper-sprayed sun leaves. However, by the last sample date, 80 days after the first spray, virtual lesions were not detected in copper-sprayed sun leaves ($P > 0.50$). Leaf spot was late to establish and significant ($P < 0.004$) virtual lesions were not detected in non sprayed sun leaves until 47 days after first being detected in copper-sprayed leaves, yet were detected on the last sample date ($P < 0.009$). Shade leaves from both treatments showed different patterns than their corresponding sun leaves. Our data suggest that copper-based fungicides can significantly impair photosynthesis early in the growing season, which may need to be considered when devising management strategies.

Genotypic diversity of *Phytophthora ramorum* in U.S. nurseries

N. J. GRUNWALD (1), M. Larsen (1), E. M. Goss (1)
(1) USDA ARS
Phytopathology 98:S63

Phytophthora ramorum is the causal agent of sudden oak death, responsible for the rapid decline of tanoak and coast live oak in California coastal forests. It also causes Ramorum blight in many common ornamentals, including *Rhododendron*, *Viburnum*, *Pieris*, *Syringa* and *Camellia*. Genetic variation in *P. ramorum* is structured into three clonal lineages, designated EU1, NA1, and NA2. EU1 is generally mating type A1 while all tested NA1 and NA2 isolates have been mating type A2. All three clonal lineages have been isolated from U.S. nurseries. We have been routinely genotyping *P. ramorum* isolates found in U.S. nurseries using microsatellite loci that exhibit variation within and between lineages. The clonal lineage of each genotyped isolate is posted to a public website along with additional information about the isolate, such as the host species and its county and state of origin (http://oregonstate.edu/~grunwaln/index.htm). We have found that NA1 continues to be the most common lineage isolated from infected nursery stock in the U.S. Our genotyping revealed the first incidence of EU1 in California in 2006, where it appeared in the same nursery as NA1. In 2007 we found the two lineages on different leaves of the same plant in an Oregon nursery. EU1 was found in all three Pacific coast states in 2007, whereas the NA2 lineage was limited to Washington State. Continued genotyping of nursery isolates will be critical for monitoring migration of the clonal lineages and the emergence of any new or recombinant lineages.

AmbR1 and AmbR2 are two transcriptional regulators essential for the antifungal activity of *Burkholderia* sp. strain MS14

G. Gu (2), S. LU (2), N. Wang (1)
(1) Citrus Research and Education Center, University of Florida, Lake Alfred, FL 32611; (2) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762
Phytopathology 98:S63

Understanding molecular mechanisms of antagonistic bacteria is important for development of biological-based strategies of plant disease management. A bacterial strain (MS14), isolated from disease suppressive soil in Mississippi, exhibited significant antifungal activities to a broad range of plant soilborne pathogens, and was characterized as a member of the *Burkholderia cepacia* complex. Random transposon and site-directed mutagenesis were conducted to identify the genes associated with its antifungal activity. Plate bioassays using an indicator fungus *Geotrichum candidum* demonstrated that a mutation in *ambR1* resulted in loss of antifungal activity, whereas an *ambR2* mutant was reduced in the activity by ~90% as compared with the wild type strain. The deduced proteins AmbR1 and AmbR2 from the two genes were 274 and 296 amino acids in size, respectively, and a predicted helix-turn-helix DNA-binding motif was identified near the C-terminus of the two proteins. AmbR1 and AmbR2 shared significant similarities to the LuxR regulatory proteins of bacteria, and had highest identities (42% and 33%, respectively) to SyrF, a transcriptional regulator, which is crucial for syringomcyin production in *Pseudomonas syringae* pv. *syringae*. Sequence analysis revealed that the two genes are located adjacent to a genomic region which contains four nonribosomal peptide synthetase genes. Mutations in three of the four peptide synthetase genes eliminated the antifungal activity of strain MS14. The regulatory functions of the two genes will be discussed in relation to expression of the genes associated with the antifungal activity.

Detoxification of Fusarium mycotoxins by microorganisms from fish digesta

S. Guan (1), J. He (1), C. Young (1), H. Zhu (1), T. ZHOU (1)
(1) Agriculture Canada, Guelph Food Research Center, 93 Stone Road West, Guelph, Ontario, N1G 5C9, Canada
Phytopathology 98:S63

The Fusarium mycotoxins, especially those belong to the trichothecene group such as deoxynivalenol (DON), are commonly found in cereal grains in Canada and frequently create significant economic losses. Since some fish species are able to live in or even favour dirty niches, there are a great variety of microorganisms harbouring in fish gut. The aim of this research was to determine if microorganisms capable of detoxifying Fusarium mycotoxins exist in the complex microbial community of fish guts. Digesta of different fishes were screened for their DON-transformation activity by being incubated in different media with pure DON. Liquid chromatography-mass spectrometry was used to determine the reduction of DON concentrations and structures of DON-transformation products. Six fish species were screened, including brown bullhead catfish (*Ameiurus nebulosus*), white sucker (*Catostomus commersonii*), largemouth bass (*Micropterus salmoides*), yellow perch (*Perca flavescens*), bluegill sunfish (*Lepomis macrochirus*) and northern pike (*Esox lucius*). Only digesta from catfish showed activity in transforming DON to de-epoxy deoxynivalenol (DOM-1), a product that is over 50 times less toxic than DON. The DON to DOM-1 transformation was 56.3%, 49.7% and 40.9% in nutrient broth, corn meal broth and full medium, respectively. No such transformation was detected in the treatments with: 1) autoclaved digesta; 2) digesta plus antibiotics, and 3) cell-free filtrates of the digesta. These results indicated that microorganisms in the tested catfish digesta were responsible for the transformation activity. They document the first DON degradation by microbes from the intestinal tract of fish. The catfish digesta will also be tested for their activity in transforming other trichothecene mycotoxins including T-2 toxin, 3-acetyl-DON and Fusarenon X. Further work will focus on isolation of the active microorganism(s) capable of degrading and detoxifying trichothecene mycotoxins.

Train-the-trainer workshops as a platform for disseminating applied nematological research to vegetable and small fruit stakeholders in the Northeast

B. K. GUGINO (1), G. S. Abawi (1), J. A. LaMondia (2), D. A. Neher (3)
(1) Cornell University, Geneva, NY 14456; (2) The Connecticut Agricultural Experiment Station, Windsor, CT 06095; (3) University of Vermont, Burlington, VT 05405
Phytopathology 98:S63

Hands-on, train-the-trainer workshops are an excellent way to extend scientific information and research results to those who interface most directly with growers and other stakeholders. Nematological outreach is especially important since the number of applied nematologists in the U.S. has been declining and fewer are being trained. To fill this void in the Northeast, a total of 13 full-day workshops supported by the NE-SARE program were developed on the diagnosis, assessment and management of plant-parasitic nematodes with a focus on the most prevalent species, *Meloidogyne hapla* and *Pratylenchus penetrans*. In addition to lecture presentations, hands-on observations of symptomatic samples and assorted nematodes under the microscope are being used to facilitate discussion. The topics covered include basic biology and ecology, diagnosis of field and crop symptoms, infestation assessment using visual on-farm bioassays and available management options (chemical, cultural and biological). In addition to a soil sampling and bioassay kit, resource materials were developed and provided in both hard and electronic formats to encourage use in outreach programming. Feedback obtained through pre- and post-workshop surveys is being used to improve future trainings and to assess project impacts. These workshops will ultimately help promote managing nematodes on an as-needed basis, thus reducing unnecessary nematicide applications and promoting the development of IPM programs.

Specific polyclonal antibodies prepared against recombinant coat protein of Pelargonium zonate spot virus and immunodetection

A. GULATI-SAKHUJA (1), H. Liu (1)
(1) USDA-ARS, Salinas, CA, USA
Phytopathology 98:S63

Pelargonium zonate spot virus (PZSV) belongs to the family *Bromoviridae*; the genus is unassigned. It was first reported in tomato plants in Southern Italy by Martelli and Cirulli (1969). PZSV was first isolated from tomato in the U.S. in 2006. Infected plants showed stunting, malformation, yellow rings and line patterns on the leaves and concentric chlorotic ringspots on the stems. The virus is transmitted by mechanical inoculation, grafting, and possibly by seed. The published PZSV coat protein sequence (GenBank Accession # NC_003651) was used to design primer pairs for cloning. Recombinant DNA technology was utilized in the production of polyclonal antibodies against coat protein (CP) gene of California isolate of PZSV. CP gene was cloned into cloning vector pTriEx4, and the protein was tagged with 6xHis at N-terminus and expressed in *Escherichia coli* cell. Over-expression of the protein was analyzed by SDS-PAGE. Recombinant protein, detected in insoluble fraction, was purified using Ni-NTA agarose resin. The purified 6xHis-PZSV-CP protein (~32 kDa) was used to immunize rabbits for raising polyclonal

antibodies. Crude antiserum was successfully used in indirect ELISA and Western blots to detect PZSV in infected tomato leaves as well as on a wide range of hosts.

Powdery mildews recently observed in Italy on ornamental plants

M. GULLINO (1), D. Bertetti (1), A. Garibaldi (1)
(1) AGROINNOVA - University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy
Phytopathology 98:S64

In recent years, severe outbreaks of powdery mildews were observed on flower crops as well as on plants used for landscape in parks and gardens. Among others, the following causal agents of powdery mildew are reported on these new hosts: *Oidium* sp. on *Akebia quinata* and *Papaver nudicaule*, *Oidium* sp. subgen. *Pseudoidium* on *Salvia scabra*, *Lonicera caprifolium*, *Mandevilla splendens*, *Berberis thunbergii* var. *atropurpurea* and *Wisteria sinensis*, *Oidium* sp. subgen. *Fibroidium* on *Euryops pectinatus*, *Oidium* sp. subgen. *Octagoidium* on *Acer negundo*, *Oidiopsis* sp. on *Asclepias curassavica*, *Erysiphe azaleae* on *Rhododendron japonicum* × *R. molle*, *Erysiphe aquilegiae* var. *aquilegiae* on *Aquilegia flabellata*, *Erysiphe orontii* on *Veronica spicata* and *Antirrhinum majus*, *Erysiphe biocellata* on *Verbena* × *hybrida*, *Erysiphe heraclei* on *Hedera helix*, *Erysiphe convolvuli* on *Ipomoea tricolor*, *Golovinomyces orontii* on *Petunia* × *hybrida* and *Lamium galeobdolon*, *Golovinomyces cichoracearum* on *Bellis perennis* and *Rudbeckia fulgida*, *Leveillula clavata* on *Euphorbia pulcherrima*, *Podosphaera fusca* on *Coreopsis lanceolata* and *Taraxacum officinale*, *Podosphaera leucotricha* on *Photinia* × *fraserii*, *Podosphaera aphanis* var. *aphanis* on *Potentilla fruticosa*, *Podosphaera spiraeae* on *Spiraea japonica*, *Podosphaera xanthii* on *Calendula officinalis.* The increased importance of powdery mildews in temperate areas could be, at least partially, attributed to the introduction of new crops as well as to the increase in temperatures observed during the spring and summer periods in the last years.

Monitoring of ochratoxin-producing *Aspergillus carbonarius* in grapevine using molecular markers

M. GULLINO (1), A. Lorè (1), K. Muthusamy (1), D. Spadaro (1), A. Garibaldi (1)
(1) AGROINNOVA - University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy
Phytopathology 98:S64

Ochratoxin A (OTA), produced on grapes by *Aspergillus* section *Nigri*, in particular by *A. carbonarius*, constitutes a threat for humans, particularly for its nephrotoxic activity. The presence of black aspergilla is a key factor in the period between veraison and harvest, causing the production of OTA in grape bunches. During 2006 a study was carried out between changing of colour of berries and harvest in two Piedmontese vineyards (cv Barbera and cv Moscato) to monitor the population of *Aspergillus* spp. Each vineyard was divided in plots and nine different chemical or biological treatments effective against B. cinerea were applied. To monitor the *A. carbonarius* population, we designed two sets of specific primers, AcPKS-F1/AcPKS-R1 and AcPKS-F4/AcPKS-R4 from a DNA sequence of a polyketide synthase (PKS) gene from *A. carbonarius* AC06. The primer sets were tested by PCR amplification on different genera of *Aspergillus*. Among the mycotoxigenic fungi viz., *A. niger*, *A. tubingensis*, *A. flavus*, *A. japanicus*, *A. aculeatus*, *A. ochraceus*, only *A. carbonarius* isolates gave amplification, confirming the specificity of both primer sets for the key ochratoxigenic species on grapes. The monitoring results showed a low level of *A. carbonarius* due to the not conducive weather conditions of the year. The study was repeated during 2007.

Antagonists' impact on enzymatic response in wilt infected cotton plants

T. G. GULYAMOVA (1), D. M. Ruzieva (1), S. M. Nasmetova (1), K. M. Karimova (1), S. M. Khodjibaeva (1), R. Stipanovic (2)
(1) Institute of Microbiology, 7B, A.Kadyri street, Tashkent, Uzbekistan, 100128; (2) USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX, USA
Phytopathology 98:S64

A number of PR-proteins possesses enzymatic activity. As such, these may be indicators of defensive response of plants. Thus, we have conducted a comparative analysis of beta-1,3-glucanase, peroxidase and xylanase activity in cotton plants to determine how these enzymes are affected by the pathogen *Verticillium dahliae* and selected antagonists (i.e., *Stachybotrys chartarum* 295, *Bacillus* sp. 234, *Streptomyces roseoflavus* 33, *Streptomyces* sp. 7 and *Trichoderma viride* 445). Analysis of enzymatic activities in the cytosol and intercellular liquid obtained from cotton plants of resistant variety Bukhara-6 revealed that enzymatic activity of intercellular liquid of leaves of control healthy plants was higher than in the cell free extracts in majority of pathogen treated plants. After infection, there was a three fold increase of glucanase and peroxidase activity, whereas xylanase activity increased by almost 10 fold. Glucanase activity was reduced in infected plants treated with antagonist and increased in uninfected plants. In contrast, peroxidase activity increased in infected plants treated with antagonist, and peroxidase activity increased in uninfected plants treated with the antagonists *S. chartarum* 295 and *Streptomyces* sp. 7. Xylanase activity increases both in healthy and infected plants on treatment with all antagonists except with antagonists *Bacillus* sp. 234 and *T. viride* 445. In general, the data reveals a positive impact of antagonists on appearance of enzymatic response in infected plants.
This work was conducted within STCU project P-226.

Serological detection of *Sweet potato leaf curl virus*

D. L. GUTIERREZ (1), P. Lotrakul (2), N. Murai (1), R. A. Valverde (1)
(1) Dept. of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, USA; (2) Dept. of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand
Phytopathology 98:S64

Sweet potato leaf curl virus (SPLCV) has been reported in sweet potato throughout the world. The virus does not normally cause symptoms in many commercial sweet potato cultivars but it can reduce yields. Virus detection methods includes: graft inoculation to indicator hosts, PCR, and molecular hybridization. Serological detection from crude sap extracts, which can be practical for diagnosticians in developing countries, is not currently available. Several attempts to purify the virus for antiserum production have failed. We tested antisera to other geminiviruses (*Bean golden mosaic virus*, *Tomato yellow leaf curl virus*, *African cassava mosaic virus*, and *Beet curly top virus*) using ELISA and Western blots, but failed to obtain cross reactivity between any of these antisera and SPLCV. In attempts to produce a fusion protein for antiserum production, primers were developed to amplify the coat protein (CP) gene of SPLCV. The CP gene was cloned into the expression vector pMAL-c2E, and the XL1-Blue strain of *E. coli* transformed.

Identification of effector genes in *Xanthomonas axonopodis* pv. *manihotis* using bioinformatics and a forward genetics screen

S. P. Gutierrez (2), L. M. Rodriguez (1), C. Pardo (2), D. Pinzon (2), N. P. Morales (2), S. Restrepo (2), A. J. BERNAL (2)
(1) Universidad Nacional de Colombia, Bogotá; (2) Universidad de los Andes
Phytopathology 98:S64

Xanthomonas axonopodis pv. *manihotis* (*Xam*) is the causal agent of cassava bacterial blight, the most limitating and devastating bacterial disease of cassava (Manihot esculenta Crantz). Like other Gram negative pathogenic bacteria, *Xam* has a type III secretion system to inject proteins into the host cytoplasm during infection. However, little is known about the effector proteins that travel through this secretion system and into the host cytoplasm. We are using two approaches to identify genes coding for effector proteins in this bacterium. In the first approach, we isolated genes by homology with reported effectors from other Xanthomonads. This was carried out experimentally using primers designed against highly conserved regions of the reported genes, or in silico, using Blast analyses against a fragmented genomic sequence of a *Xam* strain. This sequence was obtained using Solexa technology. In the second approach, we are implementing an *in vivo* genetic screen for type III effectors present in the genome. This system consists of an orphan avr gene inserted in a transposon to be used as a trap for type III secretion signals in the *Xam* genome. We have evaluated a plant-avr gene system which would be optimal for this genetic screen. We have a database of candidate effector genes present in *Xam*. The identification of effector genes will greatly contribute to the knowledge on the molecular strategies used by this bacterium to cause disease in cassava.

Simultaneous detection of *Pantoea ananatis* and *Botrytis allii* in onion seeds using magnetic capture hybridization and real-time PCR

Y. HA (1), R. R. Walcott (1)
(1) University of Georgia
Phytopathology 98:S64

Pantoea ananatis and *Botrytis allii* are seedborne plant pathogens that cause center rot and neck rot of onion respectively. To facilitate rapid and sensitive detection of these pathogens in onion seeds, magnetic capture hybridization (MCH) and multiplex real-time PCR was attempted. Biotinylated single-stranded hybridization capture probes designed to target *P. ananatis* and *B. allii* DNA were covalently attached to magnetic particles via streptavidin. Target DNA from both pathogens in onion seed samples was selectively captured using these DNA probes and amplified by multiplex real-time PCR. MCH multiplex real-time PCR displayed a detection threshold of 10 fg/µl and 1 pg/µl of *P. ananatis*, *B. allii* DNA, respectively. MCH-multiplex real time PCR was 10-fold more sensitive than direct real time PCR for the detection of *P. ananatis* (10 CFU/ml). On the other hand, similar enhancements in detection sensitivity were not observed for *B. allii* conidial suspension; however, MCH real-time PCR facilitated detection of both target pathogens in onion seed samples (n = 1,000 seeds) containing 1 *P. ananatis* infested seed and 1 *B. allii* infested seeds. These results demonstrate the applicability and

potential for MCH-multiplex real time PCR to improve simultaneous detection of multiple pathogens in onion seed lots.

Effect of mutations in HC-Pro of Soybean mosaic virus on symptom expression in soybean and the ability to induce disease synergism in mixed infection with Alfalfa mosaic virus

M. HAJIMORAD (2), A. Eggenberger (3), M. Malapi-Nelson (1), J. Hill (3)
(1) Dept. of Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN; (2) Dept. of Plant Pathology and Entomology, The University of Tennessee, Knoxville, TN; (3) Dept. of Plant Pathology, Iowa State University, Ames, IA
Phytopathology 98:S65

The potyvirus HC-Pro cistron plays multiple roles in the life cycle of potyviruses including symptom expression, but such has not been demonstrated for the Soybean mosaic virus (SMV)/soybean pathosystem. However, SMV HC-Pro has been implicated in disease synergism in soybean with Bean pod mottle virus, a property attributed to suppression of posttranscriptional gene silencing. We report here the effect of mutagenesis of HC-Pro of SMV-N (a strain G2 isolate which causes pronounced symptoms on cv Williams 82) on disease synergism in mixed infection with Alfalfa mosaic virus (AMV) in soybean and its impact on the symptom phenotype induced by SMV-N alone. Five SMV-N mutants, each containing a single amino acid substitution in the N- or C-terminal region of HC-Pro, were biolistically inoculated to fully expanded unifoliate leaves of Williams 82. The progenies were propagated in the same soybean genotype and the virions were purified and used for inoculation of Williams 82. The effects of the mutations were evaluated based upon symptom phenotype as well as the ability to mediate disease synergism in soybean co-infected with AMV strain JD. All the mutants tested induced disease synergism in mixed infection with the AMV isolate and accumulated to different levels. However, not all caused pronounced symptoms in the Williams 82 singly infected with each of the mutants. These observations suggest that alterations in HC-Pro can influence symptom expression of SMV in soybean. Furthermore, they indicate that the ability of HC-Pro of SMV to mediate disease synergism in mixed infection with AMV is distinct from its ability to induce symptoms in singly infected soybeans. The evaluation of additional SMV-N derived HC-Pro mutants and the mutagenesis of HC-Pro of SMV strain G7 (which causes mild symptoms on Williams 82) are underway.

Increase in disease impacts of *Septoria tritici* on wheat in Tunisia requires developing new control strategies

W. HAMADA (1), R. Zouid (1)
(1) National Agronomic Institute of Tunisia, 1082 Tunis, Tunisia
Phytopathology 98:S65

Septoria leaf blotch is one of the main diseases on wheat in Tunisia since in rainy seasons it could reduce yield by 50% or more. The causal agent *Septoria tritici* is known to be mainly disseminated by asexual cycle. Collected strains from field during the last season revealed the presence of both Mat1-1 and Mat1-2 indicating that sexual reproduction could occur. This could explain how strains became more severe on the main durum wheat varieties used in the country (Karim and Razek). On another hand, disease severity increased on bread wheat on which the disease was rarely observed so far. This also could be explained by some recombination events which could trigger new strains mode adapted to the bread wheat. In order to control more efficiently the disease, new developed varieties were tested to different strains. We also tried to use natural extract that could trigger defense mechanism compared to the salycilic acid and fungicide. Disease symptoms and yield components were scored from in field tests. On another hand, hydrogen peroxide was measured from plants grown under controlled conditions. Also, expression level of genes involved in general defense mechanism (PR proteins) was assessed using RT-PCR. Altogether these analysis allowed us to identify suitable varieties and treatments that could lead to better control of the disease ion the field.

Protein phosphorylation and second messenger signaling at the interface between viroid infection and symptom development

R. W. HAMMOND (1)
(1) USDA ARS MPPL, Beltsville, MD, USA
Phytopathology 98:S65

Infection of tomato by *Potato spindle tuber viroid* (PSTVd) is characterized by stunting, abnormal root and vascular tissue development, and leaf epinasty and deformation. The serine-threonine protein kinase gene *pkv*, a member of the AGC VIIIa group of signal transducing protein kinases, is transcriptionally activated in PSTVd-infected tomato plants. Constitutive overexpression of *pkv* in *Nicotiana tabacum* resulted in stunting, reduced root formation, and delay in flowering, phenotypes similar to symptoms of viroid infection. Gene expression studies suggest that gibberrellic acid biosynthetic pathways are perturbed. Our recent findings suggest that PKV second messenger signaling involves upstream interaction with and phosphorylation by 3-phospho-inositide-dependent protein kinase 1. Additional studies reveal the interaction of PKV with transcriptional activators and repressors which may function in regulating PKV-mediated cell signaling and function.

Molecular characterization of a Chinese sugar beet-infecting isolate of Beet western yellows virus

C. HAN (1)
(1) China Agricultural University
Phytopathology 98:S65

In China, sugar beet yellowing disease was reported previously by some researchers, and we confirmed that an isolate of Beet western yellows virus (BWYV) may be its agent by RT-PCR detection and partial sequences analysis in 2007. In this study, the complete genomic RNA sequence of the Chinese sugar beet-infecting isolate of Beet western yellows virus from Inner Mongolia (BWYV-IM) was determined. Results showed that the genome of BWYV-IM was 5668 nt in length, and had almost the same genome organization and characteristics with BWYV-US except the length of the intergenic non-coding region (NCR). The full length sequence of BWYV-IM had sequence identities of 86.6%, 64.4% and 70.8% with BWYV-US, Beet chlorosis virus (BChV), Beet mild yellowing virus (BMYV) respectively. Further sequence analyses indicated that the identities of all the deduced gene products between BWYV-IM and other reported beet poleroviruses were lower than 90%. According to the demarcation criteria that the differences in amino acid sequences of all the gene products were greater than 10% in the family Luteoviridae, we propose that BWYV-IM be considered as a tentative new species within the Polerovirus genus.

Interaction between weed and disease management methods in sugar beet

L. E. HANSON (2), K. A. Barnett (1), C. L. Sprague (1)
(1) Michigan State University, East Lansing, MI, USA; (2) USDA-ARS, East Lansing, MI, USA
Phytopathology 98:S65

Previous work with an experimental glyphosate-resistant sugar beet variety indicated host resistance to Rhizoctonia crown and root rot could be compromised when plants were exposed to glyphosate. In order to improve disease management recommendations, work was initiated to investigate the interaction between disease control and weed and pest management practices in commercial glyphosate-resistant sugar beet varieties. In greenhouse tests on plants treated with herbicides and subsequently inoculated with *Rhizoctonia solani* AG-2-2, statistically significant interactions were observed between sugar beet variety by herbicide spray treatment (P = 0.04) and variety by spray by the presence or absence of the pathogen (P = 0.02). A Rhizoctonia-susceptible variety showed no significant difference in disease severity regardless of herbicide treatment (alpha = 0.05), while a Rhizoctonia-resistant variety had significantly greater disease in herbicide treated plants (alpha = 0.05). Herbicides included glyphosate or a mix of phenmedipham, desmedipham, triflusulfuron methyl, and clopyralid. A variety with an intermediate response to *Rhizoctonia* showed no change in disease severity with glyphosate treatment, but had reduced disease severity when treated with standard beet herbicides (alpha = 0.05). Results indicate that disease management using host resistance may be incompatible with some weed management systems.

Efficacy of fungicides for suppressing Cylindrocladium stem and root rot of blueberry in Georgia

J. C. HARALSON (1), P. M. Brannen (1), H. W. Scherm (1), S. Nesmith (1)
(1) University of Georgia, Athens, GA, USA
Phytopathology 98:S65

Cylindrocladium stem and root rot is among the most important diseases in blueberry propagation beds in Georgia. Two trials with artificially inoculated propagation media were conducted in a shade-house on the Griffin Campus of the University of Georgia to determine the efficacy of select fungicides for control of the disease. In the first trial, a single drench application of each fungicide was made at the time the cuttings (southern highbush cultivar 'Rebel') were stuck, whereas in the second trial a second drench application was added 14 days later. In both trials, fludioxonil (Medallion) significantly reduced lesion incidence and lesion length. Neither triflumizole (Terraguard) nor thiophanate methyl (Cleary's 3336) reduced lesion incidence in trial 1, indicating that a single application of these materials was not sufficient for disease suppression. In trial 2, the second fungicide application clearly improved disease control for all fungicides. However, fludioxonil still performed statistically better than thiophanate methyl and numerically better than triflumizole as indicated by lesion incidence, lesion length, and girdling incidence. Although fludioxonil is the more efficacious product, triflumizole may be important as an alternative fungicide in a resistance management program.

Bioinformatic analysis of TonB dependent receptors of *Pseudomonas fluorescens* Pf-5

S. L. HARTNEY (1), J. E. Loper (2)
(1) Oregon State University; (2) USDA-ARS
Phytopathology 98:S66

TonB-dependent receptors (TBDRs) are outer membrane proteins that play essential roles in iron uptake by Gram-negative bacteria. The biological control strain *Pseudomonas fluorescens* Pf-5 has 45 predicted TBDRs, which far exceeds the number of TBDRs in most published bacterial proteomes. From a phylogenetic analysis of the TBDRs of Pf-5 and other *Pseudomonas* spp. (Paulsen et al. 2005), candidate TBDRs likely to function in iron-acquisition have been identified, but there are many that do not appear to function in iron acquisition. Bioinformatic analysis confirmed that all 45 TBDRs of Pf-5 have conserved domains with shared functionality, such as the plug and receptor domains. 18 TBDRs were found to have the N-terminal extension domain characteristic of transducers, a subclass of TBDRs that typically initiate a signaling pathway involving anti-sigma factors and extracytoplasmic function (ECF) sigma factors. 16 of the putative transducer genes are adjacent to genes encoding an ECF sigma factor and anti-sigma factor in the Pf-5 genome. Five of the 18 putative transducers are related to TBDRs that function in the uptake of ferric-pyoverdines; the fluorescent siderophores produced by Pseudomonas spp.; these are being tested as ferric-pyoverdine receptors in Pf-5. The remaining 27 TBDRs lack the N-terminal extension domain and linked sigma factors, but may function as receptors for a variety of substrates, as found recently in the plant pathogenic bacterium *Xanthomonas campestris* pv. *campestris* (Blanvillain et al. 2007). TonB dependent receptors may have important roles influencing the environmental fitness of the biological control strain *P. fluorescens* Pf-5.

Influence of host plant genotype on crown gall formation in walnuts

J. K. HASEY (2), M. M. Maccree (1), D. A. Kluepfel (1)
(1) USDA, Agricultural Research Service, Davis, CA, USA; (2) University of CA Cooperative Extension, Yuba City, CA, USA
Phytopathology 98:S66

Crown gall disease caused by the soil-borne bacterium *Agrobacterium tumefaciens*, seriously impacts seedling Paradox walnut rootstock grown in California. Seedling Paradox individuals with superior vigor and disease resistance traits have been micropropagated and are being tested for use in the industry. Under greenhouse conditions, we compared the following rootstock genotypes for resistance to *A. tumefaciens*: five clonal Paradox selections, Paradox seedlings, and transgenic Paradox clones modified for crown gall resistance. Three hundred microliters of a 10^7 CFU/ml suspension of *A. tumefaciens* was inoculated into a T-cut on the crown of sixteen single plant replicates per plant genotype. Plants were evaluated for gall formation up to 6.5 months post inoculation. Gall size (i.e. area) was quantified using WinRhizo software. No rootstock had zero tumors on the crown including the resistant transgenic plants where 50 percent of the individuals formed galls >6 months post inoculation. Forty-four percent of clonal Paradox 'Vlach' formed galls at the crown compared to 100 percent of seedling Paradox. Four clonal Paradox selections, 'Vlach', 'AZO25', 'RX1', and 'VX211' had galls on the crown averaging 2.6 to 4.6 cm^2 respectively. These did not differ significantly from the transgenic at 1.9 cm^2 but were significantly different from seedling Paradox (9.1 cm^2). The commercially available clonal Paradox 'Vlach' appears to have low susceptibility to *A. tumefaciens* while the new clonal releases 'VX211' and 'RX1' exhibit moderate susceptibility.

Synthetic internal control sequences to increase negative call veracity in multiplexed, quantitative PCR assays for *Phakopsora pachyrhizi*

J. S. HAUDENSHIELD (2), G. L. Hartman (1)
(1) USDA-ARS, Urbana, IL, USA; (2) University of Illinois, Urbana-Champaign, IL, USA
Phytopathology 98:S66

Quantitative PCR (Q-PCR) utilizing specific primer sequences and a fluorogenic, 5'-exonuclease linear hydrolysis probe is well established as a detection and identification method for *Phakopsora pachyrhizi*, the soybean rust pathogen. Because of the extreme sensitivity of Q-PCR, the DNA of a single urediniospore of this fungus can be detected from total DNA extracts of environmental samples. However, some DNA preparations unpredictably contain PCR inhibitors that increase the frequency of false-negatives indistinguishable from true-negatives. Two synthetic DNA molecules of artificial and arbitrary sequence were constructed, and their functionality demonstrated with the matching primers and probes as multiplexed, internal controls (ICs), to cull false-negative results by producing a positive signal that validates the PCR process within each individual reaction. The first, PpaIC, is a single-stranded oligonucleotide flanked by sequences complementary to the primers of the *P. pachyrhizi* assay itself, but targeted by a unique probe. The second, HHIC, is a dsDNA designed to utilize unique primers and probe, and was prepared as a cloned sequence in a linearized plasmid. Either IC may be added in trace amounts to raw samples before DNA extraction, or to reagent mixtures during assay set-up. Neither IC has significant similarity to natural sequences present in public databases and, whereas PpaIC will be useful mainly in P. pachyrhizi testing, HHIC has potential for use in a broad range of multiplexed Q-PCR assays when a natural internal control against false-negatives is unavailable.

Decay fungi affecting historic structures in Antarctica

B. W. HELD (3), B. E. Arenz (3), J. A. Jurgens (3), R. L. Farrell (1), S. M. Duncan (2), R. A. Blanchette (3)
(1) Department of Biological Sciences, University of Waikato, Hamilton, New Zealand; (2) Department of Bioproducts and Biosystems Engineering, University of Minnesota, St. Paul, MN 55108; (3) Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA
Phytopathology 98:S66

Decay fungi are causing serious problems in historic wooden structures and artifacts left in Antarctica from the Heroic Era of Exploration. From 1901 to 1911, Sir Ernest Shackleton and Captain Robert F. Scott, built huts on Ross Island to live and work in while attempting to reach the South Pole. On the Antarctic Peninsula, early whalers used the unique volcanic harbor of Deception Island for protection and built a whaling station comprised of many wooden structures, boats and artifacts. British and United States operations in the 1940's also resulted in a number of bases being built on the Peninsula. The historic significance of these sites has prompted conservation efforts to preserve the important Polar heritage that remains. Decay assessments made at the sites show serious biological degradation of wooden structures and artifacts is taking place. Fungi have been isolated from decayed wood samples collected from the structures and artifacts and identified by sequencing the ITS region of ribosomal DNA. Microscopy has also been used to elucidate the patterns of attack caused by these fungi. Among the Ascomycetes identified, *Cadophora* spp. are primary fungi utilizing wood substrates and causing a soft rot type of decay. *Cadophora* spp. appear indigenous to Antarctica and have been isolated in many areas, including soils. Decay causing Basidomycetes were not found except in wood from Deception Island, where a destructive brown rot as well as soft rot attack was present. These organisms appear to be important decomposer organisms in the Polar environment and further investigations will not only help to better understand their biology and ecology but provide insights for conservation to preserve these historic structures.

Characterization of sooty blotch and flyspeck fungi on pawpaw (*Asimina triloba*), a newly discovered reservoir host

K. Hemnani (1), P. O'Malley (1), B. Tanovic (2), J. Batzer (1), M. GLEASON (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA; (2) Pesticide & Environment Research Institute, Zemun-Belgrade, Serbia
Phytopathology 98:S66

Sooty blotch and flyspeck (SBFS) can cause substantial economic losses for apple growers in humid climates. Recent molecular and morphological studies indicated that the SBFS complex is comprised of more than 60 species of fungi. Fruit of pawpaw (*Asimina triloba*), a native tree in the eastern U.S., which showed signs of SBFS were sampled from non-cultivated trees in eastern Iowa. Mycelial types were examined on the fruit and on potato dextrose agar (PDA). DNA was extracted directly from mycelium on the fruit and also from pure cultures on PDA. After DNA was amplified by PCR using SBFS-specific primer set ITS-1F and Myc-1R and sequenced using ITS-1F/ITS4, nucleotide sequences were compared to those of previously identified SBFS species using the NCBI BLAST program. *Dissoconium aciculare*, *Peltaster* sp. P2.1, and *Colletogloeum* sp. FG2.2 were confirmed by matching against these species in the BLAST library. A modified Koch's on apples will be done on apple and pawpaw in 2008 to confirm our results. To our knowledge, this is the first report identifying SBFS species on pawpaw. Identifying SBFS fungi on reservoir host plants is an important step toward gaining a better understanding of the environmental biology of the SBFS complex.

Identification of *Phytophthora infestans* genes potentially involved in potato defense suppression and potentially suppressed potato genes

M. A. HENRIQUEZ (1), F. Daayf (1)
(1) Department of Plant Science, 222 Agriculture Building, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada
Phytopathology 98:S66

Late blight, caused by *Phytophthora infestans* (Mont) de Bary is a major disease of potato. New strains of *P. infestans* are resistant to fungicides and migrations of these new fungicide-resistant strains and virulent strains in the past 20 years have caused a worldwide reappearance of the potato late blight disease. In fact, our team has shown that the most aggressive strains of *P. infestans* (US-8) suppress potato defense mechanisms, through transcriptional inhibition of phenylpropanoid (PAL) and isoprenoid (HMGR) pathways.

Therefore, the objective of the current study was to identify individual cDNA fragments directly relating to pathogen genes potentially involved in potato defense suppression and potentially suppressed potato genes. Gene expression profiling was accomplished using a novel molecular approach (IDASH-Integral Differential Amplification of Subtractive Hybridization) developed for this research. IDASH was used with the *P. infestans* strains D-03 (lineage US-11, A1 mating type, low aggressiveness) and D1901 (lineage US-8, A2 mating type, high aggressiveness) inoculated to the highly susceptible cultivar "Russet Burbank", and the partially resistant cultivar "Defender". Using this approach, it was possible to select individual cDNA fragments with specific expression patterns at different times after inoculation, which showed relation with *P. infestans* cDNAs potentially involved in suppression and potato cDNAs potentially suppressed. Also, we detected *P. infestans* cDNAs potentially involved in avirulence. The validation of IDASH fragments was accomplished with Southern Blot, Northern Blot and RT-PCR. In order to get the full length cDNAs of specific fragments, a modification of the traditional RACE methodology was realized (SH-RACE) and cDNAs of 600 to 1500 bp were obtained.

Integrated management strategies for bacterial wilt on cucumbers

E. HERNANDEZ (1), P. A. Backman (1), S. J. Fleischer (1)
(1) Penn State University, University Park, PA
Phytopathology 98:S67

Bacterial wilt (BW) caused by Erwinia tracheiphila, vectored by the stripped cucumber beetle (SCB) Acalymma vittatum, is a major disease of cucumber in the northeast region of U.S. In 2007, field experiments were conducted to evaluate the integration of PGPR (BioYield™, BY), calcium silicate (CS), and phosphonate (PH) products alone, or in combination with imidacloprid insecticide (Admire 2F, IMI) or row covers, to manage bacterial wilt on cucumber. Cucumber seeds (cv. 'Tasty green') were planted into Scott's Redi-Earth transplant media in the greenhouse supplemented with BY, CS, or not. The field was drip irrigated under black plastic mulch-covered rows. The trial was a split-plot design with 6 replications of whole plots consisting of BY, BY+PH, CS, CS+ PH, PH, and no treatment. Three sub-plots were; no additional treatment, IMI, or fabric row covers. After four weeks in the greenhouse, seedlings were planted into the field. One day after transplanting, IMI was applied as a soil drench, and PH product was applied as a foliar spray on a biweekly basis. Afterward, fabric row covers were installed. Populations of SCB per plant were determined weekly. Disease incidence and severity for bacterial wilt were assessed weekly from mid-July through Sep. Cucumber fruits were harvested 6 times between Aug and Sep. Data indicates that row covers significantly reduced SCB population as well as BW, compared to IMI or untreated subplots. The combination of CS with PH, BY with PH, or BY alone in the IMI plots significantly reduced BW. Integrating row cover as a management strategy demonstrated to be as effective as IMI, increasing yield by 40%, as well as reducing the usage of IMI by 100%.

Effects of cultivars, inoculation timing, and Fusarium head blight intensity on deoxynivalenol accumulation in winter wheat

J. HERNANDEZ NOPSA (1), S. N. Wegulo (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 98:S67

Fusarium head blight (FHB), caused by *Fusarium graminearum*, can cause significant losses in wheat production. *F. graminearum* produces deoxynivalenol (DON), a harmful mycotoxin that accumulates in grain. In 2007, field experiments were conducted at Mead, NE, USA to determine the effects of cultivars, inoculation timing, and FHB intensity on DON accumulation in winter wheat. In one set of two experiments, the effect of cultivar on DON was significant (P = 0.0221). DON content in harvested grain was 1.99, 1.71, and 1.52 ppm in cultivars Jagalene, Harry, and 2137, respectively. The effect of inoculation timing (early anthesis, mid-anthesis, and natural inoculum) on DON was not significant (P = 0.7057). In a third experiment, there was a significant positive correlation between FHB severity in 11 severity categories and DON in cultivars Harry (r = 0.74; P = 0.0092) and 2137 (r = 0.70; P = 0.0157). However, DON concentration was higher in Harry (up to 4.7 ppm) than in 2137 (up to 1.7 ppm). The results from these studies indicate that (i) winter wheat cultivars differ in the levels of DON they accumulate and (ii) FHB severity is positively correlated with DON concentration.

Trunk diseases on grapevine caused by fungi in Baja California, Mexico

R. HERNANDEZ-MARTINEZ (1), C. Valenzuela-Solano (2), O. Candolfi-Arballo (1), J. A. Plata-Caudillo (1)
(1) CICESE, Ensenada, Baja California, Mexico; (2) INIFAP, Campo Experimental Costa de Ensenada. Ensenada, Baja California, Mexico
Phytopathology 98:S67

Grapevine is one of the most important crops in Baja California, Mexico. Over the last few years an increasing number of vineyards were found showing symptoms associated with trunk diseases in this region. To establish a relationship between these symptoms and fungi causing trunk diseases, a survey was carried out in the summer of 2007 in eight vineyards around Ensenada. The most common grapevine declines found were esca and black dead arm. A total of 500 spur and cordons were collected from 160 grapevines. Cross sections of infected tissue showed mainly central necrosis, brown red wood, black spotting and wedge-shaped lesions. Isolations were made on PDA with surface-sterilized wood chips. The fungus most frequently isolated from wood with dark-brown spots was *Phaeoacremonium aleophilum*, while *Botryosphaeria obtusa* was mainly isolated from wedged-shaped lesions. Other fungi isolated were *Alternaria* spp., *Fusarium* spp., and *Phomopsis* spp.

Pathology and treatment of American ginseng seed

S. N. HILL (1), M. K. Hausbeck (1)
(1) Plant Pathology Dept., Michigan State University, East Lansing, MI 48824, USA
Phytopathology 98:S67

American ginseng (*Panax quinquefolium*) is a perennial medicinal herb propagated by seeds. With an average seed yield of 336.3 kg/ha and current market prices of $55/kg, each ha represents a value of $18,537. While a number of pathogens can reduce harvest yields significantly by causing root rot and foliar blight, seed rots may also occur. Seeds may become diseased while on the plant with further infection occurring during stratification. Planting infected seed jeopardizes a grower's ability to produce a quality crop. The objective of this study was to survey seed lots from Wisconsin and Canada. *Alternaria*, *Cylindrocarpon*, *Botrytis*, *Fusarium* spp. and oomycete pathogens were obtained from the drupe tissue, seed coat and endosperm of green seed. *Alternaria*, *Cylindrocarpon* and *Fusarium* spp. were also recovered from stratified seed. In an effort to reduce *Fusarium* inoculum on seeds, fungicides and biopesticides were evaluated as treatments. Only captan consistently reduced *Fusarium* inoculum. Other products tested including sodium hypochlorite, fludioxonil, *Bacillus pumilus*, *B. subtilis*, and polyoxin D zinc salts were ineffective. Germination for all treatments was variable among replications.

***Phytophthora melonis*, *P. drechsleri* and *P. sinensis* from cucurbits**

H. H. HO (1), M. E. Gallegly (2), C. X. Hong (3)
(1) Department of Biology, State University of New York, New Paltz, NY 12561, USA; (2) Department of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506, USA; (3) Hampton Roads Agricultural Research and Extension Center, Virginia Tech, Virginia Beach, VA 23455, USA
Phytopathology 98:S67

A severe disease known as foot rot of cucumber was first described in Japan around 1948 and the causal agent was formally designated as a new species of *Phytophthora* in 1976: *P. melonis* Katsura which was characterized by homothallism, amphigynous antheridia, semipapillate sporangia and the production of chlamydospores. Since then a smililar disease recognized as dieback, root rot or crown rot, was reported on cucumber and other cucurbits in mainland China, Taiwan, Iran, Egypt, Turkey, Korea and India. Isolates from all these countries were similar to *P. melonis* except that the sporangia were non-papillate and proliferated internally; also, chlamydospores were not found. The latter isolates have been identified as *P. melonis*, *P. drechsleri* and *P. sinensis* sp. nov. Yu & Zhuang whereas Ho (1986) considered them all conspecific based comparative morphological studies under similar cultural conditions. However, data based on the ITS sequence analysis of ribosomal DNA have demonstrated that *P. melonis* is a genetically distinct species, different from *P. drechsleri* but identical with *P. sinensis*. We have repeated the study by conducting single-strand conformation polymorphism analysis (SSCP) of rDNA and confirmed the previous molecular findings. To avoid further nomenclature confusion, we have thus revised the morphological description of *P. melonis* to change the sporangia from semipapillate to nonpapillate and delete the statement regarding the presence of chlamydospores. With this ameneded species description, all isolates from cucurbits previously identified as *P. drechsleri* or *P. sinensis* should be renamed as *P. melonis* which is conspecific with but has priority over *P. sinensis*.

Aerial content and viability of *Monilinia fructigena* conidia in relation to brown rot development and weather factors in environmentally-benign apple production system

I. J. HOLB (1)
(1) University of Debrecen, Centre of Agricultural Sciences, P.O. Box 36, H-4015 Debrecen, Hungary
Phytopathology 98:S67

In a 3-year Hungarian study, aerial spore content of *Monilinia fructigena* conidia, brown rot development and weather variables were monitored from

mid-May until harvest on cultivar Mutsu in integrated and organic apple orchards. Spore viability was also determined. Seasonal concentrations of conidia were 5–10 times higher in the organic orchard than in the integrated one. Conidia of *M. fructigena* were first trapped in late May in both orchards in all years. Number of conidia greatly increased after the appearance of first infected fruit and increased continuously until harvest. Final brown rot incidences were 4.3–6.6% in the integrated and 19.8–24.5% in the organic orchards. A significant relationship described by separate three-parameter Gompertz functions was found between disease incidence and corresponding cumulative numbers of trapped conidia in both orchards. Conidia caught over a 24-h period showed distinct diurnal periodicity in both systems, with peak concentration between 13.00 and 18.00 hours. Percent viability of conidia ranged from 48.8 to 70.1% with lower values in the dry than in the wet days in both orchards and all years. Temperature and relative humidity correlated best with mean hourly conidia catches in both orchards in each year. Correlations between aerial conidia content and wind speed were significant only in the organic orchard over the 3-year period. Mean hourly rainfall negatively correlated with mean hourly conidia catches.

Combining sanitation practices with timing of scab sprays in organic apple production

I. J. HOLB (1)
(1) University of Debrecen, Centre of Agricultural Sciences, P.O. Box 36, H-4015 Debrecen, Hungary
Phytopathology 98:S68

The aim of this study was to evaluate scab control efficacy in integrated approaches of i) three sanitation treatments (fallen leaf removal combined with winter pruning and non-sanitized control), ii) three onsets of first fungicide sprays (dormant bud, early tight cluster and pink bud stage), and iii) three final dates for finishing fungicide programs (mid-July, mid-August and mid-September) in an organic apple orchard on two cultivars. Results on scab resistant cultivar Prima indicated that sprays against scab could be omitted before pink bud stage and after mid-July by holding the threshold of 1% fruit scab incidence. On moderately scab susceptible cultivar Jonathan, a delay in the onset of first spray until pink bud stage resulted in higher scab incidences on both leaves (16.6–20.6%) and fruits (13.1–15.3%) compared with the non-delayed spray treatments (5.2–8.3% and 6.7–9.4%, respectively). Final leaf and fruit scab incidence increased significantly when sprays were omitted after mid-July compared to spray treatments finished at mid-August or mid-September. A combination of leaf removal with pruning resulted in lower scab incidence (5.2–11.8%) compared with the non-sanitized plots (6.7–14.7%) when spray treatments were finished at mid-August or mid-September. Results on cultivar Jonathan suggested that sprays against scab could only be omitted before early tight cluster stage and after mid-August if leaf removal combined with pruning was applied.

Molecular characterization of *Monilinia fructicola* populations with different sensitivities to DMI fungicides

I. J. HOLB (2), P. K. Bryson (1), G. Schnabel (1)
(1) Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC 29634, USA; (2) University of Debrecen, Centre of Agricultural Sciences, P.O. Box 36, H-4015 Debrecen, Hungary
Phytopathology 98:S68

Severe economic losses may occur in stone fruit production due to the emerging resistance to sterol demethylation inhibitor (DMI) fungicides in *Monilinia fructicola*. The genetic diversity of seven *M. fructicola* populations with differing levels of field exposure and sensitivities to DMI fungicides was assessed to characterize these populations on a molecular level. Two populations with no previous exposure to DMI fungicides (baseline populations), and five populations with long-time DMI exposure (commercial populations) were studied. Isolates from two of the five commercial populations were resistant to the DMI fungicide propiconazole. The 14-alpha demethylase (*MfCYP51*) gene, encoding the target enzyme for DMI fungicides, was sequenced for 6 sensitive and 4 resistant isolates in search for nucleotide variations. A variable restriction site was used to conduct polymerase chain reaction - restriction fragment length polymorphism analysis of the *MfCYP51* gene. Additionally, inter-simple sequence repeat analysis of total genomic DNA was performed. Results indicated larger genetic variation in baseline populations compared to DMI resistant isolates. Among commercial orchards, DMI-resistant populations revealed less genetic diversity compared to DMI-sensitive populations. Results suggest that extensive fungicide exposure reduces the genetic variation and diversity of *M. fructicola* populations, likely due to the selection of resistant strains.

Using wikis to communicate plant pathology information

G. J. HOLMES (1), B. A. Edmunds (1), S. J. Colucci (1)
(1) NC State University, Raleigh, NC, USA
Phytopathology 98:S68

Wikis are a type of collaborative software that allows web pages to be created and edited using a web browser. Wikis were introduced to the general public in 2001 by the success of Wikipedia. Other wikis have been developed specifically for the purposes of cooperative extension (collaborate.extension.org/wiki) and pest management (wiki.bugwood.org) and have a more restricted policy regarding authorship. Access to information is increasingly defined not by presence on the Internet, but by ranking on search engines. Wikipedia offers maximum accessibility because of the large user base and high profile for search engines. It provides a rapid method for getting information out with maximum exposure while allowing real-time editing. The validity of information on Wikipedia is sometimes questioned, but problems are most common with controversial current events. Deliberate tampering with plant pathology information is not likely. One downside to most wikis for plant pathologists is the lack of authorship which is important in documenting individual efforts. However, this can be offset somewhat by hyperlinking text to other websites and photos where authorship is clear. Currently Wikipedia contains "stubs" (very short entries) for most plant diseases. We used Wikipedia to rapidly publish high-quality, effective fact sheets that were immediately detected and highly ranked by popular search engines.

Water quality dynamics in irrigation runoff retention basins and its practical implications for plant health management

C. HONG (3), P. Richardson (3), S. Ghimire (3), P. Kong (3), G. Moorman (1), J. Lea-Cox (2), D. Ross (2)
(1) The Penn State; (2) University of Maryland; (3) Virginia Tech
Phytopathology 98:S68

Irrigation runoff water retention basins as an aquatic ecosystem are increasingly important in plant health management locally and agricultural biosecurity globally. Irrigation water is an important source of inoculum for a variety of destructive plant pathogens and an efficient means of pathogen dissemination. The primary objectives of this study were to monitor water quality dynamics and assess its potential impacts on survival of pathogens returning to retention basins through irrigation runoff. The status of (i) chlorophyll a, (ii) conductivity, (iii) dissolved oxygen, (iv) oxidation-reduction potential, (v) pH, (vi) salinity, (vii) temperature, (viii) total dissolved solids, and (ix) turbidity were recorded hourly using a Hydrolab DataSonde 5X in a retention basin where water was sampled regularly for *Phytophthora* species. Monitoring was done beginning in November and extended for 6 months. Dramatic changes in water pH, dissolved oxygen, and oxidation-reduction potential were observed to be closely associated with algal blooms. Both pH and dissolved oxygen concentration fluctuated coincidentally, whereas oxidation-reduction potential changed inversely with chlorophyll readings. A peak in turbidity followed each algal bloom. Total dissolved solids, salinity and conductivity also peaked after each algal bloom event, but they tended to increase over time. The practical ramifications of these observations are discussed in relation to developing of novel water decontamination technology and plant health management in general.

***Phytophthora irrigata* and *Phytophthora hydropathica*, two new species from irrigation water at ornamental plant nurseries**

C. HONG (3), M. Gallegly (4), P. Richardson (3), P. Kong (3), G. Moorman (1), J. Lea-Cox (2), D. Ross (2)
(1) The Penn State; (2) University of Maryland; (3) Virginia Tech; (4) West Virginia University
Phytopathology 98:S68

Two subgroups within the *Phytophthora drechsleri* complex, previously (2003) referred to as "Dre I" and "Dre II", are provisionally named as *Phytophthora irrigata* and *Phytophthora hydropathica* respectively. Like *P. drechsleri*, these two new species are heterothallic, produce nonpapillate sporangia, and grow well at 35°C. However, they differ significantly from true *P. drechsleri* by size of sporangia, pattern of DNA fingerprint and sequence of ribosomal DNA internal transcribed spacers (rDNA-ITS) as well as ecological niche. They both produce plerotic oospores and each produces a very distinct DNA fingerprint. Their closest relatives are *P. insolita* and *P. polonica*. The optimal temperature for culture growth is about 30°C and the temperature maximum is 40°C. They are abundant in irrigation reservoirs during the warm summer. These two new species also differ from each other. *P. hydropathica* produces chlamydospores and obovate hyphal swellings whereas *P. irrigata* produces none. The former produces larger sporangia than the latter (55 by 39 vs. 44 by 33 micrometer). They also differ genetically as indicated by DNA fingerprint and sequence analyses of rDNA-ITS. In addition, *P. hydropathica* has been recovered from necrotic leaves and blighted shoots of *Rhododendron catawbiense* as well as from stem base of wilting *Kalmia japonica* plants randomly collected at nurseries where water was surveyed but *P. irrigata* has not been isolated from any plants. The potential impacts of these two new species on horticultural crops as well as natural forests will be discussed.

Oxylipins act as quorum sensing signals and cell density regulators in *Aspergillus* spp.

S. HOROWITZ BROWN (2), R. Zarnowski (1), J. B. Scott (2), W. C. Sharpee (2), N. P. Keller (2)
(1) Department of Medical Microbiology and Immunology, UW Madison; (2) Department of Plant Pathology, UW Madison
Phytopathology 98:S69

Aspergillus flavus differentiates to produce asexual conidia or overwintering survival structures called sclerotia. Results here show both processes are oppositely regulated by density dependent mechanisms where increasing cell density (from 10^1 to 10^7 cells) yields lowest sclerotial and highest conidial numbers. Production of most secondary metabolites, including aflatoxin, follows the same trend as sclerotial production. These density dependent phenomena can be affected by lipid availability. Addition of linoleic acid increased sclerotial formation in intermediate cell densities (10^4 to 10^5). Furthermore, disruption of a putative oxylipin generating gene, *Aflox* encoding a lipoxygenase, greatly diminished density dependent development of both sclerotia and conidia with an overall increase in sclerotia and decrease in conidia at high cell densities (>10^5 cells). This is reminiscent of studies in the model fungus *A. nidulans* where cleistothecial-conidial balance is governed by oxylipins (oxygenated unsaturated fatty acids). In addition to *Aflox*, analysis of the *A. flavus* genome has led to the identification four other oxylipin generating oxygenases (*ppoA-D*). Transcriptional profiling of *ppo* and *Aflox* shows each has a distinct expression pattern during *A. flavus* development as might be predicted for proteins generating signaling molecules. These accumulating data supported a hypothesis that lipid pools, particularly oxylipins, may be involved in quorum driven cell density transitions in *Aspergillus*.

Aggressive strains of the wheat yellow rust fungus spread world-wide

M. HOVMØLLER (1), A. H. Yahyaoui (2), E. A. Milus (3), A. F. Justesen (1)
(1) Aarhus University, Faculty of Agricultural Sciences, Denmark; (2) International Center for Agricultural Development in the Dry Areas, P.O. Box 5466, Aleppo, Syria; (3) University of Arkansas, Department of Plant Pathology, Fayetteville, AR 72701, USA
Phytopathology 98:S69

Recent research has shown that different strains of the biotrophic wheat yellow rust fungus, *Puccinia striiformis* f. sp. *tritici*, vary significantly in aggressiveness, i.e., the ability to cause severe disease rapidly on a susceptible wheat cultivar. Based on DNA-fingerprints of more than 150 representative single-lesion *P. striiformis* isolates sampled from a large number of wheat cultivars on five continents and epidemiological observations of yellow rust epidemics in these areas, we demonstrate that two closely related (differed by only two of polymorphic DNA fragments), aggressive strains spread to North America, Australia and Europe in less than three years. The new strains became rapidly widespread across the two former continents, while resistant cultivars have so far prevented widespread epidemics in Europe. The new aggressive strains, which were also observed in West- and Central Asia and East Africa, showed only limited divergence in virulence phenotype, irrespective of the cultivar from which isolates were obtained. This further supports a hypothesis of very recent spread, taking into account the high rate of mutation in virulence phenotypes. The data also gave evidence for additional intercontinental *P. striiformis* dispersal events in the past.

A new PVY strain from Idaho: An NTN recombinant which causes no veinal necrosis in tobacco

X. HU (1), T. Meacham (1), L. Ewing (1), A. V. Karasev (1)
(1) University of Idaho, Moscow, ID 83844-2339
Phytopathology 98:S69

A novel PVY strain (L26-2) was isolated from a Frontier potato line submitted to the University of Idaho tissue culture lab for a standard clean-up procedure. This strain was initially typed as an NTN recombinant using a RT-PCR based protocol by Lorenzen et al. (2006), and confirmed to be a recombinant by a limited sequencing. In ELISA, L26-2 reacted with N-specific antibodies, like a normal NTN strain. However, in a biological assay, after inoculation of tobacco plants, this PVY strain caused mosaic and mild vein clearing, and did not induce vein necrosis characteristic of regular NTN or other recombinant strains tested side-by-side with L26-2. We amplified and sequenced the HC-Pro region of the L26-2 genome. Both "signature" amino acid residues, thought involved in induction of the veinal necrosis syndrome in tobacco, K-400 and E-419 (Tribodet et al., 2005), were found present in the HC-Pro of L26-2. The data obtained suggest that the veinal necrosis genetic determinant of PVY in tobacco is likely complex and includes other element(s), in addition to the C-terminal fragment of HC-Pro.

Gene flow of *Phytophthora infestans* between organic and conventional potato field in Southern Flevoland, The Netherlands

C. HU (1), F. Govers (2), J. Ristaino (1)
(1) NCSU, Raleigh, NC, USA; (2) Wageningen University, Wageningen, The Netherlands
Phytopathology 98:S69

Phytophthora infestans causes a devastating disease of potato and tomato. *P. infestans* can reproduce asexually and sexually; and both mating types have been detected in several European countries since the early 1980s. Previous work in the Netherlands showed that population is highly diverse due to sexual reproduction and that oospores can overwinter in the soil. In Southern Flevoland, the genotypic diversity was analyzed by characterizing isolates from conventional and organic potato fields, potato refuse piles, and allotment gardens for mating type and DNA fingerprints between 1994 and 1996. Refuse piles were identified as the most important infection source for commercial fields in 1994 and 1995 and some organic fields were identified as a source of mid-season infection of conventional fields; however, only RFLP fingerprint patterns were used for the analysis. We sampled DNA from these populations and amplified two regions of a single-copy nuclear ras gene (intron 1 -224 bp and exon 3-6 -542 bp) containing both coding and noncoding regions to elucidate gene flow between organic and conventional fields. Multiple isolates were sampled from 5 organic, 17 conventional and 7 refuse pile sites. There were 5 heterozygous sites in the intron 1 region and 5 heterozygous sites in the exon 3-6 region that were phylogenetically informative. Only two isolates from an organic and conventional field were homozygous. Five haplotypes were found among the fields and three of these were found in our previous world wide study of Andean migration. Further analyses of additional mitochondrial gene regions are underway to investigate the migration of *P. infestans* between these fields.

Identification of anthracnose fungi by heteroduplex mobility assay and heteroduplex pattern

T. HUANG (2), Y. Yeh (1), D. Tzeng (2)
(1) Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Taipei, Taiwan; (2) Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan
Phytopathology 98:S69

Colletotrichum spp. are the casual agents of anthracnose on various crops and the post-harvest pathogens greatly deteriorating the quality and shelf-life of agricultural products. Identification of *Colletotrichum* spp. by morpho-taxonomic criteria is often frustrating and time consuming because these pathogens have wide host range, vary in morphological characteristics with environment, and cross infect on one host. To cope with these pitfalls, the application of heteroduplex mobility assay of ITS regions as a biochemical tool was explored. The ITS regions of 29 *Colletotrichum* isolates including *C. gloeosporioides*, *C. acutatum*, *C. musae*, *C. graminicola*, *C. capsici*, *C. dematium*, *C. lindemuthianum*, and *Colletotrichum* sp., were PCR amplified and subjected for HMA. The data from HMA of ITS regions suggested that 29 *Colletotrichum* isolates could be divided into 5 distinctive species groups. The correlation of DNA distance and relative heteroduplex mobility observed among 15 reference isolates was used to construct a formula for estimation of distances of a tested DNA sequences from relative heteroduplex mobility. The phylogenetic analysis of ITS regions of 29 *Colletotrichum* isolates using DNA distance inferred from relative heteroduplex mobility concluded them into five groups similar to that concluded from DNA sequences analysis. Analysis of the heteroduplex pattern (HP) with reference isolates *C. gloeosporioides* Cg1, *C. gloeosporioides* Cg16, *C. musae* Cm1, *C. gloeosporioides* Cg2, *C. graminicola* Cgr1 and *C. lindemuthianum* Cl1 indicated that isolates of *C. gloeosporioides* could be further divided into two subgroups whereas the ITS regions varied only in six bases. In conclusion, HMA and HP of ITS regions are relative rapid and convenient methods for species-specific identification of *Colletotrichum* spp.

Development of microsatellite markers for *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-lycopersici*

C. HUANG (1), P. D. Roberts (2), L. E. Datnoff (1)
(1) University of Florida, Gainesville, FL, USA; (2) University of Florida, SWFREC, Immokalee, FL, USA
Phytopathology 98:S69

Florida is probably the center of origin of several vegetative compatibility groups (VCGs) of *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), causing Fusarium wilt and Fusarium crown rot on tomato, respectively. Microsatellite markers were developed for population genetic studies due to their high polymorphism. Microsatellite loci were either identified from a genome sequence of FOL by a tandem repeat finding program or isolated from FORL by a microsatellites enrichment procedure. Thirty-eight loci were derived from the bioinformatics approach and 14 loci were acquired from the enrichment procedure. Length

polymorphisms were evaluated from representative VCGs of FOL and FORL. Of 52 original loci, 26 were amplified consistently and varied for the VCGs examined. Interestingly, a primer pair CH1-29, did not amplify VCGs 0030-0033 of FOL but amplified VCGs 0094, 0098, and 0099 of FORL, which were predominant in Florida. This indicates the possibility of using this primer to differentiate FORL from FOL. These 26 microsatellite markers are being used, along with the VCG assay, for population genetic studies of FORL.

Chemical composition and antifungal activity of the fruit essential oil of star anise (Illicium verum Hook f)

Y. Huang (1), L. ZHOU (1)
(1) China Agricultural University
Phytopathology 98:S70

Star anise (Illicium verum Hook f) is a medium-sized evergreen tree, distributed in the tropical and subtropical zones of Asia. The fruits are frequently used as a famous spice in food industry, and are also used as traditional medicine in treatment of stomach and pain diseases in eastern Asia. The fruit essential oil, obtained by hydro-distillation, was investigated by gas chromatography-mass spectrometry (GC-MS). Trans-anethole (83.483%), limonene (2.403%), 2-(1-cyclopentenyl)-furan (0.894%) and cis-anethole (0.708%) were found to be the major components among 29 identified components. The antifungal activities of the oil against 11 kinds of plant pathogenic fungi were determined by mycelial radial growth inhibition method. The values of the mean inhibitory concentration (IC50) against the tested fungi were at a range of 0.07 μg/mL to 0.25 μg/mL. Furthermore, IC50 of the essential oil against spore germination of Magnaporthe oryzae was determined to be 0.32 μg/mL. The volatile components of the oil also obviously inhibited the mycelial growth of Pythium aphanidermatum and Botryodiplodia theobromae. The results indicate that star anise essential oil could be a potential antifungal agent for plant (especially for fruit and vegetable post-harvest) disease control.

Population studies of a newly introduced species of *Raffaelea* causing laurel wilt disease in the southeastern United States

M. A. HUGHES (3), J. A. Smith (4), A. E. Mayfield (1), R. Ploetz (5), S. W. Fraedrich (2), T. J. Dreaden (4)
(1) Florida Department of Agriculture and Consumer Services, Division of Forestry, Gainesville, FL, USA; (2) USDA Forest Service, Southern Research Station, Athens, GA, USA; (3) University of Florida, Department of Plant Pathology, Gainesville, FL, USA; (4) University of Florida, School of Forest Resources and Conservation, Gainesville, FL, USA; (5) University of Florida, Tropical Research and Education Center, Homestead, FL, USA
Phytopathology 98:S70

Redbay (*Persea borbonia*) a tree native to the southeastern United States is being devastated by laurel wilt disease, caused by an undescribed species of *Raffaelea* that is vectored by the exotic redbay ambrosia beetle (*Xyleborus glabratus*) and affects other species in the Lauraceae. In 2003 the disease was first observed in southern South Carolina and around Savannah, Georgia, but the disease has since spread rapidly inland and southward into the Florida peninsula. Previous studies have not addressed the genetic diversity and population structure of this pathogen. Preliminary results from inoculation experiments of avocado (*Persea americana*) suggest differences in virulence among isolates. Subsequent analysis using randomly amplified polymorphic DNA (RAPD) primers revealed genetic differences between these isolates. The genetic diversity of 60 *Raffaelea* isolates recovered from infected hosts in FL, SC, and GA are being analyzed to identify potential heterogeneity and structure in the pathogen population. Implications for future work to assess disease resistance in host species and additional epidemiological studies will be discussed.

Yield of soybean inoculated with two genotypes of the brown stem rot causal agent, *Phialophora gregata* f. sp. *sojae*

T. J. HUGHES (1), N. C. Koval (1), C. R. Grau (1)
(1) University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 98:S70

Brown stem rot (BSR) is a yield limiting disease of soybean (SB) in the North Central United States. In 2000, two genotypes of the causal agent, *Phialophora gregata* f. sp. *sojae* (Pgs), were identified; A (PgsA) and B (PgsB). Previous studies indicate isolates of PgsA are more aggressive than isolates of PgsB and SB cultivars resistant to BSR are preferentially colonized by PgsB. Current information regarding the yield impact of BSR have inadvertently focused on loss caused by isolates of PgsA. The objective of this project was to assess the yield of SB cultivars inoculated with PgsB. Field microplots (1 m^2) were established in a location never planted to SB. Seed of eight SB cultivars, four resistant and four susceptible, were planted into furrows containing vermiculite mixed with PgsA, PgsB, PgsA and B, or vermiculite alone. BSR severity was assessed at R7 and yield determined at R8. Experiment was conducted for 2 years and once in the greenhouse. Treatments with PgsB tended to decrease yields compared to vermiculite alone across all cultivars. However, treatments with PgsB resulted in statistically lower yields in only the second field year ($P = 0.003$). In addition, yields of BSR-resistant LL89-605 and BSR-susceptible Newton and Williams were consistently lower for treatments with PgsB in both field and greenhouse experiments. Real-time qPCR assays will be used to assess population of PgsA and B in SB stems to determine correlation between yield and colonization.

Variation in population density and diversity of *Phytophthora* species in streams within a forest watershed

J. HWANG (1), S. W. Oak (2), S. N. Jeffers (1)
(1) Dept. of ESPS, Clemson University, Clemson, SC, USA; (2) USDA Forest Service, Southern Region, FHP, Asheville, NC, USA
Phytopathology 98:S70

Water samples from a portion of the Davidson River watershed in western North Carolina were collected and assayed to help determine the number of sample sites needed to effectively survey watersheds for *Phytophthora* species. The sampled watershed covers 32.6 km^2 and consists of nine sub-watersheds, each drained by an individual stream that runs into the Davidson River. Seven streams, each in a separate sub-watershed, and the drainage point at the lower end of the Davidson River were sampled twice, in Sep and Oct 2007. Samples (1 liter) from all streams were collected within a 30-min period to minimize variation that may be associated with time of day. Nine 100-ml aliquots were filtered from each sample and filters were inverted onto PARPH-V8 selective medium; colonies of *Phytophthora* spp. were counted after 3 days at 20C, and numbers of colony-forming units (cfu) were calculated. Densities of *Phytophthora* spp. were lower in the streams draining the upper sub-watersheds. In Oct, the mean density of *Phytophthora* spp. from the three upper streams was 5 cfu/liter while a mean density of 52 cfu/liter occurred in three lower streams. Six known species--*P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. heveae*, and *P. pseudo-syringae*--and three previously unidentified species were recovered from streams in the watershed. The diversity of species varied among streams and was greater in Oct than in Sep. In Sep, five species were detected within the watershed and three of these were recovered at the lower drainage point. In Oct, nine species were detected in the watershed and five of these were recovered at the drainage point.

Construction of a virus-induced gene silencing (VIGS) vector for cotton using *Cotton leaf crumple virus* and a fragment of the cotton phytoene desaturase gene

A. M. IDRIS (2), B. Ktenz (3), J. R. Tuttle (1), H. Jeske (3), D. Robertson (1), J. K. Brown (2)
(1) Department of Plant Biology, North Carolina University, NC; (2) Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA; (3) Universitaet Stuttgart, Biologisches Institut, Stuttgart, Germany
Phytopathology 98:S70

Cotton leaf crumple virus (CLCrV) is a bipartite begomovirus native to the southwestern U.S.A., Mexico, and Guatemala. Previously we cloned the genome of CLCrV and demonstrated infectivity and reproduction of characteristic disease symptoms in cotton *Gossypium hirsutum* seedlings. The CLCrV DNA-A component was modified by deleting a fragment of the coat protein gene (Cp) to create an 'attenuated' virus-induced gene silencing (VIGs)-vector backbone into which foreign sequences can be inserted. The 'VIGs vector backbone' was co-inoculated with the unmodified DNA-B component into highly susceptible 'Delta Pine (DP) 5415' cotton plants by biolistic inoculation. Inoculated plants were systemically infected, but disease symptoms were attenuated. A 340-bp fragment of the cotton (*G. hirsutum*) phytoene desaturase (Gh-PDS) gene was RT-PCR amplified from 'DP 5415'. The Gh-PDS fragment was inserted into the 'VIGs backbone' to create a construct that when inoculated to cotton plants was expected to silence cotton Gh-PDS. The Gh-PDS VIGs vector was mixed with an equal amount of CLCrV DNA-B and co-bombarded into 'DP 5415' seedlings. The plants were held at in a growth room under a 16-hr day: 8-hr night light regime at 28°C. Phytoene desaturase plays a role in carotenoid synthesis and therefore silencing of this gene manifested as white bleaching, that was evident 8–12 days post-inoculation in the inoculated leaves. Thereafter, silencing became systemic, and symptom intensity continued to intensify. Subsequently, plants were transferred to a greenhouse in which the temperature ranged from 16°C at night to 26°C during the daytime. Under these conditions the Gh-PDS VIGS induced complete bleaching of all subsequently developing leaves. Wild type disease symptoms were observed both in cotton plants inoculated with the CLCrV VIGs backbone and the DNA-B component, and with the unmodified, wild type CLCrV. These results indicate that altered environmental conditions (possibly, temperature and/or light) may affect the plant innate defence system, which in these experiments was expressed as variable silencing of Gh-PDS.

An approach to restore sexuality in *Fusarium oxysporum*
S. Imai (1), T. Teraoka (1), T. ARIE (1)
(1) Tokyo University of Agriculture and Technology (TUAT), 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan
Phytopathology 98:S71

In heterothallic ascomycete fungi, isolates of opposite mating type are required to initiate the sexual cycle. Mating-type of each isolate is determined by the *MAT1* locus carrying one of two highly divergent alleles known as idiomorphs that are designated *MAT1-1* or *MAT1-2*. *Fusarium oxysporum* is considered to be an asexual ascomycete lacking a sexual phase in its life cycle. However, *MAT1* idiomorphs were identified in the *F. oxysporum* genome which were homologous to those in *Gibberella fujikuroi*, a sexual relative of *F. oxysporum*, and the *MAT1* genes were expressed. A recent phylogenetic study of *F. oxysporum* f. sp. *lycopersici* (*FOL*), the tomato wilt fungus, revealed three well-supported clades, each of which contained isolates of only one mating type. Moreover, whole-chromosome PFGE analysis revealed that electrophoretic karyotypes were highly variable among clades. These data suggest that *F. oxysporum* may be unable to complete the sexual cycle due to chromosomal divergence among clades and lack of a compatible mate within each clade. To address this hypothesis, we generated a transformant of *FOL* in which the *MAT1-2* idiomorph was replaced with the *MAT1-1* idiomorph. The electrophoretic karyotype of the transformant was identical to that of the parental isolate. When the original isolate (*MAT1-2*) and the transformant (*MAT1-1*) were paired under mating conditions of *G. fujikuroi*, unknown structures which did not show up in wild type were observed.

Microscopic characterization of the pathogenic phase of *Phialophora gregata* in soybean stems
A. E. IMPULLITTI (1), D. K. Malvick (1)
(1) University of Minnesota, St. Paul, MN
Phytopathology 98:S71

Brown stem rot (BSR) is a soybean disease caused by two genotypes of the fungal pathogen *Phialophora gregata* (*Pg*). *Pg* type A (*Pg*-A) causes necrosis and chlorosis of leaves and internal stem necrosis, and *Pg*-B primarily causes stem necrosis. The objective of this study was to elucidate the interaction between soybean and *Pg*-A and *Pg*-B using light microscopy. Plants were inoculated with both genotypes, stems were collected 5 weeks later, and the mid-section was cross sectioned and stained. The average cross sectional area of individual vessels (N = 1400 vessels), and average vessel area colonized by *Pg* in a susceptible (Corsoy 79) and a resistant (Bell) cultivar were analyzed using digital images. In the susceptible (S) cultivar, 44% and 37% of the vessel area was colonized by *Pg*-A and *Pg*-B, respectively. In addition, there were 45% fewer vessels and the average vessel area was 15% greater for infected plants than non-inoculated plants. The greater vessel area of the S cultivar infected with *Pg* allows more colonization by *Pg*-A. In the resistant (R) cultivar, 6% and 43% of the vessel area was colonized by *Pg*-A and *Pg*-B, respectively. The area of colonized and non-colonized vessels was similar in R plants inoculated with *Pg*-A. The non-inoculated R cultivar had 50% greater vessel area than plants inoculated with *Pg*-A. R cultivars infected with *Pg* may decrease vessel area as a defense response to limit pathogen movement. Investigations utilizing *Pg*-A and *Pg*-B tagged with fluorescent proteins are also underway to provide further resolution of the *Pg* – soybean interaction.

High diversity of *Botryosphaeria* species from almond band and canopy cankers in California
P. INDERBITZIN (1), T. J. Michailides (1)
(1) UC Davis, Kearney Ag Center, Parlier, CA
Phytopathology 98:S71

Band canker is a common and serious fungal disease affecting almonds in California. Whereas band canker affects the trunks and main scaffolds, a different disease, canopy canker, appeared in 2004. Canopy canker affects smaller branches throughout the tree. The causal agent for both diseases was identified as *Botryosphaeria dothidea*, based on morphology. As a pathogen with a wide host range, *B. dothidea* is also known from natural vegetation and agricultural crops routinely growing in close proximity to almond orchards. The goal of this study was to determine the origins and inoculum sources of the *B. dothidea* strains causing canopy canker. We used phylogenetic analyses based on five different genetic loci, and more than 140 *B. dothidea* isolates from band and canopy cankers, as well as other plant hosts near almond orchards. We found that canopy canker isolates were genetically diverse, falling into five different groups. These were *B. dothidea*, two groups close to *Neofusicoccum parvum* (formerly *B. parva*), *N. mediterraneum*, as well as one group close to *N. mediterraneum*. Virulence assays by inoculating almond branches in the field showed that isolates close to *N. parvum* were most virulent compared to *B. dothidea* and *N. mediterraneum*. All groups of canopy canker isolates also contained band canker isolates and/or isolates from other hosts such as walnut and blackberry that might constitute potential inoculum sources. Our study suggests that besides *B. dothidea*, other, possibly new species might play prominent roles in band and canopy canker diseases.

Development of potato black dot from seed-tuber born inoculum
J. INGRAM (1), D. Johnson (1)
(1) Washington State University
Phytopathology 98:S71

The rate of spread of *Colletotrichum coccodes*, the cause of potato black dot, was investigated on the below ground parts of Russet Burbank potato plants. Greenhouse grown plants arising from seed-tubers with natural and inoculated *C. coccodes* infection were observed. Beginning 21 days after plant emergence and continuing at 14 day intervals, the distance that *C. coccodes* colonized potato lateral roots was recorded using destructive sampling. Distance was measured from the seed-tuber to the distal edge of sclerotial development along the lateral roots. Visual assessment of disease progress was confirmed by re-isolation from diseased and non-diseased tissues. Expansion of *C. coccodes* foci along the lateral roots in two trials resulted in a linear growth model of 1 mm/day disease progress ($P < 0.01$, R^2 0.83). Colonization always radiated from the seed-tuber to form compact foci of infected tissue; no separate, isolated or secondary foci were observed. These results suggest that *C. coccodes* expands linearly from a source of inoculum and that for multiple disease foci to develop on roots, multiple primary infections must occur from over wintering sclerotia in the soil. This linear disease progress would suggest that management tactics should be explored for reducing initial inoculum effects for potato black dot on roots, below ground stems and tubers.

Dose response of soilborne plant pathogens and *Meloidogyne incognita* to citrus-based experimental compounds
F. B. IRIARTE (1), E. N. Rosskopf (1), N. Kokalis-Burelle (1)
(1) USHRL, ARS, USDA, Fort Pierce, FL, USA
Phytopathology 98:S71

Two novel citrus-based compounds have been tested *in vitro* against *Colletotrichum gleosporioides*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Verticillium albo-atrum*, *Pythium aphanidermatum*, *P. myriotilum*, *Phytophthora nicotianae* and *P. capsici*. One of the compounds, referred to as Citroxin (aka Oraphyte), contains hydrogen peroxide and the other compound, Citroxin O2 (aka Oraphyte O2) does not. Both compounds were effective in controlling all fungi tested at concentrations ranging from 1 to 4%. The formulation containing hydrogen peroxide was more effective for controlling *C. gleosporioides*, *V. albo-atrum*, *F. oxysporum*, *P. nicotianae*, and *P. myriotilum*, while Citroxin O2 was more effective for *S. sclerotiorum* and *S. rolfsii*. No significant difference in percent kill was found for *R. solani*, *P. aphanidermatum* or *P. capsici* up to 2%, after which higher concentrations of Citroxin were more effective than Citroxin O2. IC50's ranged from 0.6 to 1.3% for Citroxin, and 0.6 to 2% for Citroxin O2. *Meloidogyne incognita* juvenile nematodes were killed *in vitro* with a concentration of 1% of both formulations. In addition, egg hatch was inhibited at 1% with Citroxin and 2% with Citroxin 02. In greenhouse experiments, a post-transplant drench of 25% Citroxin provided control of Phytophthora blight of pepper. In tomato, the same concentration significantly reduced the number of root-knot nematode galls and number of eggs per gram of root without causing phytotoxicity.

Diverse bacterial plant pathogens contain homologs of the *Xanthomonas oryzae* pv. *oryzicola avrRxo1* effector gene
H. ISHIHARA (1), B. Zhao (2), J. E. Leach (1)
(1) Colorado State University, Ft. Collins, CO, USA; (2) Virginia Tech, Blacksburg, VA, USA
Phytopathology 98:S71

The maize NB-LRR gene *Rxo1* confers a hypersensitive response (HR) and induction of defense response genes after challenge with the rice bacterial streak pathogen *Xanthomonas oryzae* pv. *oryzicola*. *X. o.* pv. *oryzicola*, which is not a pathogen of maize, encodes a type III secretion-dependent effector *avrRxo1*. Four additional homologs of *avrRxo1* were cloned from *Burkholderia andropogonis*, *Acidovorax avenae* subsp. *avenae*, *A. a.* subsp. *citrulli*, and *X. axonopodis* pv. *vesicatoria*. While the amino termini of the homologs are diverged, the carboxy termini are similar, with the *Xanthomonads* grouping into one cluster, and the *Burkholderia* and *Acidovorax* grouping into a second cluster. The amino acid similarities between AvrRxo1 homologs ranged from 49% to 86%. All five AvrRxo1 homologs contain a putative ATP/GTP binding site; mutation of this site in the *X. o.* pv. *oryzicola avrRxo1* eliminates HR function. The five homologs are adjacent to an ORF with similarity to chaperones; the two ORFs have GC contents distinct from the genomes of each source bacterium, suggesting that

they were horizontally acquired. While all *X. o*. pv. *oryzicola* strains from Asia contain *avrRxo1* and exhibit a strong HR in maize and rice with *Rxo1*, only some strains of *B. andropogonis* contain the homolog and elicit an HR on *Rxo1*-containing plants. Almost all strains of *A. avenae* and *X. a*. pv. *vesicatoria* contain the *avrRxo1* homolog, but only *A. avenae* subsp. *avenae* elicited an HR on *Rxo1*-plants. The presence of *avrRxo1* homologs in diverse bacterial pathogens indicates that the gene might play a role in pathogenic fitness/aggressiveness.

Signaling and interactions between plants and phytopathogenic Peronosporomycetes

M. ISLAM (1), A. Tiedemann (1)
(1) Division of Plant Pathology and Plant Protection, Georg-August-Universitaet Goettingen, Goettingen, Germany
Phytopathology 98:S72

The Peronosporomycetes genera such as *Phytophthora*, *Plasmopara*, *Aphanomyces*, *Pythium* produce characteristic bi-flagellated motile zoospores, where motility is considered an important means of pathogen distribution and infection of the plants. Zoospores of these pathogens precisely locate their infection sites guided by the host-specific chemical signal(s) and then rapidly undergo a series of morphological changes before penetration. Some host-specific plant signals (e.g., cochliophilin A, prunetin, indole-3-carbaldehyde, daidzein, genistein) involved in recognition and pre-infectional development of zoosporic pathogens (*Aphanomyces* and *Phytophthora*) have been identified in last two decades. We found that the rapid morphological changes of zoospores (e.g., *Aphanomyces cochlioides*) on host surface induced by host-specific signaling compounds (e.g., 5-hydroxy-6,7-methylenedioxyflavone) are linked to dynamic polymerization/ depolymerization of the filamentous actin in their cells. Although the detail signal transduction pathways of zoosporogenesis, chemotaxis and subsequent differentiation of zoospores are yet to be elucidated, our research revealed that these processes are initiated by G-protein-coupled receptors, which activate phosphoinositide and Ca^{2+} second messenger pathways. In contrast, diverse secondary metabolites such as nicotinamide (motility inhibitor and regenerator), stilbenes, saponins, anacardates and polyflavonoid tannins (motility inhibiting and lytic factors), alkaloids (inhibitor of tubulin assembly), and isoflavonoids (repellants) in nonhost plants were found to affect/interfere specific stage(s) of the life cycle of phytopathogenic Peronosporomycetes, and are likely to be responsible for their incompatibility. This talk will focus our recent findings on chemistry and biological activities of the host and nonhost secondary metabolites and their potential role implicating interactions of *Aphanomyces cochlioides* and *Plasmopara viticola* zoospores with plants.

Zoosporogenesis and differentiation of grapevine downy mildew pathogen *Plasmopara viticola* in host-free system

M. ISLAM (1), A. Tiedemann (1)
(1) Division of Plant Pathology and Plant Protection, Georg-August-Universitaet Goettingen, Goettingen, Germany
Phytopathology 98:S72

Plasmopara viticola is an obligate biotrophic pathogen of downy mildew disease in grapevine. The objective of this study was to unravel mechanisms of asexual sporulation and early development of this peronosporomycete phytopathogen. We developed convenient methods to produce copious amount of sporangia and zoospores (*ca.* 10^6 ml^{-1}) following asexual life stages (cystospore, germinated cysts) of P. viticola in host-free systems. These reproducible methods facilitated investigations of mechanisms of asexual sporulation and differentiation of zoospores by using pharmacological tools (agonist/antagonists) commonly used in cell biology. We tested compounds activating G-protein (mastoparan) and antagonizing protein kinases (K-252a and KN-93), calcium channels (verapamil), calmodulin (trifluoroperazine), phospholipase C (U-73122), and transcription (actinomycin D) for their effects on zoospore release, motility, and differentiation of zoospores. The verapamil and the trifluoroperazine inhibited zoosporogenesis and encystment. The protein kinase inhibitors K-252a and KN-93 inhibited zoospore release and cyst germination, and K-252a significantly reduced motility of zoospores. Concentrations of actinomycin D did not affect zoospore release or encystment. The encystment of zoospores was triggered by both phospholipase C (PLC) and the G-protein activator mastoparan in a dose dependent manner. However, U-73122 did not completely block mastoparan induced encystment but suppressed PLC induced encystment suggesting that not only PLC but also phospholipase D (PLD) pathways are active in encystment process. Altogether, these results show that P. viticola contains a mastoparan-inducible PLC and/or PLD pathways with a strong indication that PLC is involved in zoospore encystment. Proteomic analysis for characterizing stage-specific proteins, and quantification of lipid metabolism and enzyme activities for understanding signal transduction pathway in zoospore encystment are in progress.

Isolation and characterization of soil bacteria capable of detoxifying the mycotoxin deoxynivalenol (DON)

M. R. ISLAM (2), J. He (2), P. P. Pauls (1), T. Zhou (2)
(1) Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada; (2) Guelph Food Research Center, AAFC, 93 Stone Road West, Guelph, Ontario, Canada, N1G 5C9
Phytopathology 98:S72

Fusarium graminearum (Schwabe) produces deoxynivalenol (DON) in cereals, which poses a serious health threat to humans and animals. Due to the limitation of available methods for detoxifying mycotoxins in food and feeds, we screened soil samples for DON degrading microorganisms. Soil samples collected from various locations in Ontario, Canada were inoculated with 100 ppm DON and analyzed by High Performance Liquid Chromatography (HPLC) for DON degradation ability. We investigated the effects of five factors namely, inoculum size, growth medium, pH, incubation time, and temperature on biodegradation of the mycotoxin. Among the tested soil samples, one was able to degrade DON completely (100% degradation) on mineral salts broth (MSB) after incubation for 3d at pH 7 and 25°C. However, degradation was observed only at high microbial population density. To facilitate the isolation of the target bacteria, the microbial mixture was sub-cultured in MSB amended with antibiotics. After several transfers of the culture in MSB treated with 100 ppm penicillin, the microbial diversity was determined by Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis. T-RFLP fingerprints showed a significant reduction of microbial diversity in the enriched culture compare to initial soil samples without affecting the DON degradation activity. From Liquid Chromatography-Ultraviolet–Mass Spectrometric (LC-UV-MS) analysis, the degraded product was identified as de-epoxy DON (also known as DOM-1), which is significantly less toxic than DON. Isolation of the active bacteria using the serial dilution plating method is in progress. Following isolation, the bacteria will be characterized by gram-staining, oxidase testing, cell surface observation and 16S-rRNA gene analysis. This study will contribute to the identification of a novel source of DON detoxifying gene(s). Cloning of this bacterial gene(s) and its stable integration into a cereal genome could potentially reduce severity of *Fusarium* diseases and minimize the toxicity of DON in *Fusarium* infested cereals.

Diversity of sooty blotch and flyspeck fungi from Serbia and Montenegro

M. Ivanovic (3), J. Latinovic (1), N. Latinovic (1), J. C. Batzer (2), K. Hemnani (2), M. L. GLEASON (2)
(1) Biotechnical Institute, University of Montenegro, Podgorica, Montenegro; (2) Department of Plant Pathology, Iowa State University, Ames, IA, USA; (3) University of Belgrade-Zemun, Belgrade, Serbia
Phytopathology 98:S72

Sooty blotch and flyspeck (SBFS) fungi cosmetically damage apple fruit by forming dark spots and blemishes on the fruit surface. The SBFS complex was long believed to comprise just two species, but more than 60 putative species have now been identified in the eastern U.S. In 2007, apples infested with SBFS were collected from 16 localities in Serbia and two in Montenegro. Signs on fruit were isolated onto acidified water agar and purified on potato dextrose agar. Mycelium was scraped from pure cultures and DNA was extracted, amplified, and sequenced using the primer set ITS-1F/ITS-4. Morphological observations of conidia and signs on apple fruit suggest that the most commonly occurring SBFS colonies were caused by *Pseudocercosporella* spp. Fungi within the genera *Zygophiala* and *Peltaster* were also observed. Preliminary evidence suggests that there are some SBFS fungi that are common to northern Europe and North America, but there appear to be other species that may be unique to the Balkan region.

Identifying *Phytophthora* species isolated from stream baits in North Carolina

K. L. IVORS (1), M. D. Greene (1)
(1) Dept. of Plant Pathology, North Carolina State University, Fletcher, NC, USA
Phytopathology 98:S72

Ten locations throughout North Carolina were initially established as part of the National *Phytophthora ramorum* Early Detection Survey in Forests. Non-wounded *Rhododendron* leaves were placed in mesh bags as baits and secured in perennial watercourses for seven to fourteen day intervals. Recovered symptomatic leaves collected from May through November 2007 were plated on selective media. Putative *Phytophthora* isolates were provided to our lab from the North Carolina Department of Agriculture and identified using ITS sequencing. Isolation of *Phytophthora* species varied by location and time of sampling. This collection of over 100 isolates included multiple representatives of the four described species *P. gonadopodyides*, *P. citricola*, *P. citrophthora* and *P. cryptogea*, and eight clades of previously undescribed *Phytophthora* species. Future work will include additional stream monitoring

and ITS identification of isolates, as well as addressing the survival, spread and significance of these undescribed species in North Carolina watercourses and natural ecosystems.

Modification of seed exudates by seed-colonizing microbes from vermicompost alters pre-infection behavior of *Pythium aphanidermatum* zoospores

A. H. JACK (1), E. B. Nelson (1)
(1) Cornell University, Department of Plant Pathology and Plant Microbe Biology, Ithaca, NY, USA
Phytopathology 98:S73

Suppression of plant diseases with composts is well documented, but the microbial mechanisms involved are poorly understood. For diseases caused by Pythium spp., the spermosphere is a critical habitat for microbial interaction and host infection, leading us to hypothesize that seed colonizing microbes from composts may have important impacts on compost-mediated disease suppression. To test this hypothesis, we established the suppressiveness of vermicomposted dairy manure (VC) in cucumber bioassays with *Pythium aphanidermatum* zoospore inoculum. Seed and seedling health were significantly improved with VC amendments. However, sterilized VC did not provide protection, indicating the observed suppression is biological in nature. Transplant experiments were conducted to establish the temporal pattern of seed colonization and disease suppression. These experiments revealed that *P. aphanidermatum* zoospores were able to colonize/infect seeds within 27 hours of sowing in unamended soil. Vermicompost microbes colonizing seeds within 24 h of sowing were incubated in the presence of seed exudates to obtain microbially-modified seed exudates (MMSE). When given a choice between different exudates, fewer zoospores encysted on vermicompost MMSE than on unmodified exudates, indicating that these exudates were less attractive to zoospores. This result shows that, within a short time frame, seed colonizing VC microbes can interfere with the zoospores' ability to respond to exudates. Interference with one or more zoospore pre-infection events including; attraction, encystment, attachment and germination, is proposed as a potential mechanism for vermicompost-mediated suppression of *P. aphanidermatum* on cucumber.

Identifying differences in gene expression between Race 1 and Race 3 strains of *Ralstonia solanacearum* during bacterial wilt disease development at warm and cool temperatures

J. M. JACOBS (1), F. Meng (1), C. Allen (1)
(1) University of Wisconsin, Department of Plant Pathology, 1630 Linden Dr., Madison, WI 53706
Phytopathology 98:S73

The plant pathogenic bacterium *Ralstonia solanacearum* elicits wilt disease on many hosts, causing significant losses for farmers worldwide. One group in this species complex, Race 3 biovar 2 (R3bv2), persists and causes disease at moderately cool temperatures, in contrast to tropical *R. solanacearum* strains. Genome sequences of R3bv2 strain UW551 and tropical Race 1 biovar 3 strain GMI1000 suggest about 10% of UW551 ORFs are not present in GMI1000; these may explain R3bv2's temperate epidemiology. UW551 and GMI1000 behave similarly i*n vitro* at cold (4C), cool (20C) and warm (29C) temperatures, but UW551 is nevertheless much more virulent on tomato plants at 20C than GMI1000. This result suggests that we must study UW551 gene expression *in planta* to understand how it causes wilt disease at cooler temperatures. These phenotypic observations and the genomic differences between strains will frame a comparative gene expression analysis of GMI1000 and UW551 during infection of tomato plants at 20C and 29C. We therefore designed microarrays representing the GMI1000 and UW551 genomes and developed methods for extracting quality bacterial RNA from infected tomato plants. This powerful four-way comparison of two different strains and temperatures should suggest the molecular mechanisms that govern R3bv2 cold tolerance.

Survival and histopathology of eastern white pine seedlings from controlled crosses infected with *Cronartium ribicola*

J. J. JACOBS (1), T. A. Burnes (1), J. A. Smith (2), C. Sweeney (3), A. J. David (4), R. A. Blanchette (1)
(1) Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA; (2) School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA; (3) U.S. Forest Service - Oconto River Seed Orchard, White Lake, WI, USA; (4) University of Minnesota, North Central Research and Outreach Center, Grand Rapids, MN, USA
Phytopathology 98:S73

White pine blister rust caused by *Cronartium ribicola* is an extremely damaging non-native disease of five-needled pines in North America. Recent efforts to reduce damage caused by this fungus have focused on identification and propagation of disease free eastern white pine (*Pinus strobus*) growing in areas with a high incidence of blister rust. In this study, 13 eastern white pine families derived from controlled pollination of trees previously determined to possess putative resistance were inoculated with *C. ribicola*. Mortality was documented and needle infections were collected for histological examination. Superior survivability of crosses P327xP312, P327xP327 and P327xON469 was evident with more than 60% of seedlings from each family surviving the 52 week monitoring period compared to 0% survival for the most susceptible cross. Histological examination of needle infections from these crosses often revealed deposition of phenolic compounds around the infection site and collapse of mesophyll cells. Differences in relative size of infection zone within needles of cross P327x327 and more susceptible crosses were also documented. Results suggest that resistance mechanisms in the needle can be variable among seedlings from different crosses. In many sections, haustoria and hyphae were observed in the vascular bundle far from the origin of infection apparently uninhibited by host responses. The continued characterization of resistance, selection of trees with superior defense mechanisms and their utilization in breeding programs for reforestation and urban tree planting have the potential to significantly mitigate future damage caused by white pine blister rust in North America.

A horizontally acquired cellulose synthase operon in *Dickeya dadantii* contributes to biofilm formation and attachment to plants

C. E. JAHN (1), J. Apodaca (2), N. T. Perna (2), A. O. Charkowski (1)
(1) Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, USA; (2) Genome Center of Wisconsin, University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 98:S73

Enterobacterial animal pathogens exhibit aggregative multicellular behaviour, which manifests as pellicles on culture surfaces and biofilms at the surface-liquid-air interface. In *Escherichia coli* and *Salmonella enterica*, cellulose contributes to pellicle formation and its synthesis requires four operons: *bcsABZC*, *bcsEFG*, *adrA*, and *csgD*. Homologues of *bcsABZC* and *adrA* were identified in the *Dickeya dadantii* genome, but organization of the operons differs from even the most closely related sequenced enterobacteria. *D. dadantii* also lacks *bcsEFG* and *csgD*, yet still produces cellulose. In rich media, *D. dadantii* forms pellicles that can be degraded by cellulase. The *bcsA* mutant forms pellicles that are morphologically distinct in scanning electron micrographs and are not degraded by cellulase. The *adrA* mutant produces cellulose-containing pellicles similar to wild-type, indicating that *adrA* is not essential for cellulose synthesis in *D. dadantii.* This result, combined with the likely horizontal gene transfer of the *bcs* operon, suggests alternate regulatory and cellulose synthesis mechanism(s) compared to other enterobacteria. Mutations in *bcsA* or *adrA* did not inhibit the growth of this pathogen *in planta*. Interestingly, a 30-fold increase in DNA uptake was observed in the *adrA* mutant. The *bcsA* mutation was reduced in ability to attach to alfalfa sprouts, whereas the *adrA* mutation was increased in attachment to plant tissue. Cellulose may have a role in the survival of this organism by protection from antimicrobials. After exposure to 1% bleach, there was a significant reduction in survival of the *bcsA* mutant while 100% of the wild-type cells survived.

Formulations of *Aspergillus flavus* AF36 to improve in-field residence and sporulation

R. JAIME-GARCIA (2), P. J. Cotty (1)
(1) USDA-ARS, University of Arizona, Tucson, AZ; (2) University of Arizona, Tucson, AZ
Phytopathology 98:S73

Aflatoxins, toxic and carcinogenic *Aspergillus* metabolites, are frequent contaminants of crops including cottonseed. Contamination can be reduced by applying atoxigenic strains of *A. flavus*. Applications of atoxigenic strain product (sterile wheat colonized with strain AF36) are considered successful if 80% or more of the aflatoxin-producing fungi are displaced by a single application of 10 lb/acre. However, some cotton fields treated in Arizona fall below this range because much of the product is lost before sporulation and dispersal. The quantity of product in the field two weeks after application is positively correlated with displacement of aflatoxin producers. Field observations indicate that product loss is mainly caused by animal feeding. Formulations that deter feeding and/or lead to earlier and better sporulation will favor dispersal of the applied strains and improved displacement of aflatoxin producers. The objective of the present study was to develop new formulations that reduce loss of both product and product viability. Formulations were evaluated in commercial cotton fields. Results indicate that coating of the colonized wheat seed with either chili powder or colorants allows for better product persistence without influencing product sporulation. Replacing wheat with sorghum as the carrier and fungal nutrient source provides both longer in-field residence and greater sporulation.

Mitogen-activated protein kinase cascade in signaling polyamine biosynthesis in tobacco

E. Jang (2), K. Min (2), S. Nam (3), S. Zhang (1), Y. Kim (2), B. Cho (2), K. YANG (2)
(1) Department of Biochemistry, University of Missouri-Columbia, Columbia, MO 65211, USA; (2) Department of Plant Biotechnology, Chonnam National University, Gwangju 500-757, Korea; (3) Jeonnam Agricultural Research & Extension Services, Jeonnam, Naju, 520-715, Korea
Phytopathology 98:S74

Expression of *NtMEK2*^{DD}, a constitutively active mutant of NtMEK2, activates endogenous SIPK and WIPK and leads to several stress/defense responses in tobacco. In this study, we used ACP (annealing control primer)–based differential display RT-PCR to isolate the downstream effectors mediated by the NtMEK2-SIPK/WIPK cascade. Arginine decarboxylase gene (*ADC*), which is involved in plant polyamine biosynthesis, was one of 10 differentially expressed genes. When compared with *NtMEK2*^{KR} plants, *NtMEK2*^{DD} transgenic plants exhibited a significant increase in *ADC*, *ODC* and *MPO1* transcript levels, as well as in the putrescine and spermine content following SIPK/WIPK activation. Taken together, these results suggest that the NtMEK2-SIPK/WIPK cascade regulates polyamine synthesis, especially putresine synthesis, through transcriptional regulation of the biosynthetic genes in tobacco.

Yeast microflora of nectarines

W. J. JANISIEWICZ (1), C. Kurtzman (2)
(1) Appalachian Fruit Research Station, ARS-USDA, Kearneysville, WV, USA; (2) NCAUR, ARS-USDA, Peoria, IL
Phytopathology 98:S74

Resident fruit microflora has been the source of biocontrol agents for control of postharvest decays of fruits, and the active ingredient in commercialized biocontrol products. Except grapes and apples, information on the resident microflora of other fruits is only fragmentary, but greater knowledge in this area can be very helpful in developing biocontrol. We characterized the yeast microflora of nectarines (cv. 'Croce del Sud') from the early stages of fruit development until harvest. The fruit samples were collected from trees in an unmanaged orchard block. The resident fruit microflora was separated from the occasionally deposited microorganisms by discarding initial fruit washings before the final wash, that was follow by sonication and plating on NYDA medium. The isolated yeasts were identified by BIOLOG and by sequencing the D1/D2 domain of a large subunit of the rRNA gene, and where available, the ITS sequence. BIOLOG identified 19 and the genetic analysis 13 species of yeasts. Although the identification by these two systems was not always the same, the predominant yeasts were *Rhodoturula* spp., *Sporodiobolus* spp., *Cryptococcus* spp., *Pichia* spp. and *Candida* spp. Some of the identified yeast species were previously shown to control postharvest decays on different fruits.

Verticillium wilt resistance in U.S. potato breeding programs

S. H. JANSKY (1)
(1) USDA-ARS
Phytopathology 98:S74

Verticillium wilt is a serious disease that causes annual yield loss in most potato production regions in North America. It is most commonly caused by the soil-borne fungal pathogen *Verticillium dahliae*. The only consistently effective control practice is fumigation. Therefore, host plant resistance offers an attractive alternative control method. High levels of resistance are not present in most commercially significant cultivars, although 'Ranger Russet' is considered to be moderately resistant. In a two year study, 11 cultivars and 14 advanced breeding clones from throughout the U.S. were evaluated for resistance to *V. dahliae*. They were planted into infested soil and evaluated for symptom expression (AUDPC), fresh stem colonization (colony forming units per ml plant sap) and dried stem colonization (cfu per g dry stem). Significant differences were detected for clone, year, and the clone by year interaction. Dried stem counts were higher in 2006 than in 2007, while sap counts were higher in 2007 than in 2006. The most resistant clones in both years included the cultivar 'Megachip' and four clones from three potato breeding programs. This study indicates that good progress is being made toward the incorporation of Verticillium wilt resistance into potato breeding programs.

Impact of preceding crops on incidence and severity of diseases in canola

B. M. Jenks (1), S. A. MAZUREK (1), G. P. Willoughby (1)
(1) North Dakota State University - North Central Research Extension Center, Minot, ND 58701
Phytopathology 98:S74

Recent announcements for canola biodiesel production in North Dakota have increased interest in growing canola in ND. Biodiesel plants may require increased canola production in the state. Many small grain producers in the northern plains have turned to producing alternative broadleaf crops. Current recommendations are to plant a broadleaf crop like canola or sunflower no more than once every four years to avoid buildup of disease inoculum. Additional information on the impact of crop rotation on disease would help producers optimize their limited resources. A twelve-year rotation study was initiated at the NDSU-NCREC in Minot, ND in 2000 to determine the impact of preceding crops on disease incidence and severity in canola. Six rotations were evaluated and every phase of the rotation is present every year in a randomized complete block design replicated four times. The rotations consist of canola every one, two, three, or four years preceded by canola, flax, or wheat. Half of each canola plot was treated with fungicide to prevent Sclerotinia stem rot (SSR). Plots were evaluated for SSR risk; SSR and blackleg incidence and severity; and yield and test weight. The objectives of this research were to: document the influence of crop rotation on the incidence and severity of sclerotinia, and blackleg in canola; determine the impact of the previous crop on disease levels in canola; and determine if fungicide applications can be avoided by altering the sequence of crops in the rotation. To date, general observations on disease risk and incidence indicate it is more dependent on environment than rotation. In general, blackleg incidence has increased from 2000 to 2006 across all rotations in the study. However, blackleg severity has remained relatively low. This may be because only canola varieties rated moderately resistant or resistant have been used in the study. Both blackleg incidence and severity increase as the frequency of canola in the rotation increases. Differences between rotations or fungicide treatments for both sclerotinia and blackleg have been documented, but to date are too small to determine significant differences.

Clp mediates signal transduction for xylanase and motility, not for biofilm formation in *Xanthomonas oryzae* pv. *oryzae*

K. JEONG (1), G. Kim (1), W. Kim (2), J. Cha (1)
(1) Dept. of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea; (2) Honam Agricultural Research Institute, RDA, Iksan 570-080, Korea
Phytopathology 98:S74

To find function of Clp in *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which is known as global regulator for expression of virulence factor in *Xanthomonas campestris* pv. *campestris* (*Xcc*), marker exchange mutant of Clp homolog was generated. Virulence, xylanase activity and motility of the mutant were reduced whereas EPS and cellulase activity were not. Virulence, motility, and xylanase activity of the mutant were restored to wild type strain's level by complementation. When expression of the 14 genes that reported as virulence factor in *Xoo* by RT-PCR, expression of gene for phytase (*phy*A), xylanase (*xyn*B) and motility (*pil*A) were significantly decreased in the Clp mutant while genes for EPS (*gum*G and *gum*M), LPS (*xan*A, *xan*B, *wxo*D and *wxo*C), cellulase (*egl*2 and *cls*A), cellobiosidase (*cbs*A), lipase (*lip*A) and iron metabolism (*fur*) were not. Expression of the genes for EPS and LPS were not decreased in the mutant inoculated in rice leaf while expression of all other genes was decreased. Expression of *clp* gene were decreased in the mutants of RpfC and RpfG that were reported as components of two-component system transferring signal from DSF (diffusible signal factor) to virulence genes in *Xcc*. These results suggest that Clp transfers DSF signal from Rpf C/RpfG to some of the virulence genes including for motility and xylanase, but it is not involve in regulation of genes for the biofilm formation in *Xoo*.

TIP is required for basal resistance but not for HRT-mediated hypersensitive response or resistance to Turnip crinkle virus in Arabidopsis

R. JEONG (2), A. C. Chandra-Shekara (2), A. Kachroo (2), D. Klessig (1), P. Kachroo (2)
(1) Boyce Thompson Institute, Cornell, Tower Road, Ithaca, NY, USA; (2) University of Kentucky, Lexington, KY, USA
Phytopathology 98:S74

Inoculation of Turnip crinkle virus (TCV) on the resistant Arabidopsis ecotype Di-17 elicits hypersensitive response (HR), accompanied by increased expression of defense genes. HR to TCV is conferred by HRT, which encodes a coiled coil-nucleotide binding-leucine rich repeat class of resistance (R) protein. HR to TCV is initiated upon perception by HRT of the avirulence factor, the viral coat protein (CP). The CP also interacts with a member of nuclear-targeted NAC family of host transcription factors, designated TIP (TCV-interacting protein). Binding to the viral CP prevents nuclear localization of TIP and retention of TIP in the cytosol has been proposed to activate the HRT-mediated pathway. To further investigate the relationship between TIP and HRT, we screened for plants carrying homozygous knockout mutation in the TIP gene and assayed these for HR and resistance to TCV. The HRT tip plants showed normal basal defenses and induced normal HR upon TCV inoculation. These plants were also resistant to TCV. Furthermore, the mutation in TIP neither impaired the salicylic acid-mediated induction of HRT expression nor the enhanced resistance conferred by overexpression of HRT. Interestingly, the mutation in TIP allowed increased accumulation of

TCV and Cucumber Mosaic Virus. Together, these results suggest that TIP is required for basal resistance but not for binding HRT-mediated HR or resistance.

Development of the recombinant inbred line population of tropical *Japonica* Lemont crossed with *Indica* Jasmine 85

Y. JIA (1), G. Liu (2), A. McClung (1)
(1) USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR; (2) University of Arkansas Rice Research and Extension Center, Stuttgart, AR
Phytopathology 98:S75

A recombinant inbred line (RIL) population of rice is useful for studying agronomically important genes and analyzing quantitative trait loci (QTL) since genotypes are stable and can be assessed over years. Jasmine 85, a midseason aromatic long-grain *indica* rice cultivar, developed at the International Rice Research Institute, Philippines, is resistant to the common U.S. races of *M. oryzae*, IA45, IB1, IB49, IC17, ID1, IG1 and IH1, susceptible to IB54; resistant to *Rhizoctonia solanii*, *Cercospora oryzae*, and *Entyloma oryzae*, but susceptible to 'straighthead' - a physiological disorder. Lemont, an early maturing, semi-dwarf tropical *japonica* cultivar commercially grown in the southern U.S. from 1980's to 1990's, is resistant to blast races IA45, IB45, IB54, IG1 and IH1, but susceptible to IB1, IB49, IC17, to *C. oryzae* and *R. solani*. A total of more than 800 RILs of the cross of Lemont with Jasmine 85 have been advanced to the F8-9 generation using single seed descent in a greenhouse. A subset of 256 RILs was genotyped using 199 simple sequence repeat (SSR) markers, and a high density SSR linkage map has been constructed representing a total of 1684.2 cM of genetic distance, averaging 8.5 cM between markers. This mapping population should be useful for the rice scientific community for identifying genes for a number of disease resistance and agronomic traits. Limited seed can be obtained by writing to Dr. Yulin Jia (Yulin.Jia@ARS.USDA.GOV) at Dale Bumpers National Rice Research Center, Stuttgart, AR.

A region-wide analysis of genetic diversity in *Verticillium dahliae* infecting olive in Andalusia, southern Spain

R. M. JIMENEZ-DIAZ (3), C. Olivares (3), J. A. Navas-Cortes (1), B. B. Landa (1), M. Jimenez-Gasco (2)
(1) CSIC, Cordoba, Spain; (2) The Pennsylvania State University, University Park, PA, USA; (3) University of Cordoba, Cordoba, Spain
Phytopathology 98:S75

Verticillium wilt, caused by *Verticillium dahliae*, has become the main threat for the Andalusia olive industry. Severity of attacks by *V. dahliae* is associated with the spread of a highly virulent, defoliating (D) pathotype of vegetative compatibility group 1A (VCG1A). Genetic diversity in 637 *V. dahliae* isolates from 72 olive orchards in the five most important olive-growing provinces in Andalusia was studied by VCG typing using *nit* mutants of the international OARDC reference strains and local testers, molecular pathotyping using specific PCR primers, and sequence analysis of a 539/523-bp *V. dahliae*-specific PCR amplicon. VCG1A (77.8%), VCG2A (20.5%) and VCG4B (1.5%) were identified among 636 isolates and one isolate was heterokaryon self-incompatible. VCG1A isolates were of the D pathotype. VCG1A was the predominant VCG in four provinces. A single VCG prevailed among isolates within an orchard, but the association VCG1A/VCG2A occurred in 11 orchards. A large proportion of VCG1A isolates occurred in orchards established with un-rooted olive sticks, irrigated with underground water, or planted in virgin soil or soil previously cropped to non-hosts. Only three (*seq1*, *seq2*, and *seq4*) of the seven known sequences (*seq1* through *seq7*) of the *V. dahliae*-specific amplicon were identified among the isolates in the study. VCGs and *seq* sequence distribution in the sampled orchards suggest that genetic diversity within *V. dahliae* populations is higher in provinces were VCG1A is not prevalent. The D pathotype dominates the *V. dahliae* population in the olive-growing area of southern Spain, against which there is no resistance available.

New polymorphic markers for analysis of genetic diversity in *Verticillium dahliae* populations

M. JIMENEZ-GASCO (1), M. Berbegal (2), J. Yanez (1), C. Garzon (4), J. Armengol (2), R. M. Jimenez-Diaz (3)
(1) The Pennsylvania State University, University Park, PA, USA; (2) Universidad Politécnica de Valencia, Valencia, Spain; (3) University of Cordoba, Cordoba, Spain; (4) Wooster College, Wooster, OH, USA
Phytopathology 98:S75

Verticillium dahliae is an, asexually-reproducing, soilborne fungus that causes vascular wilt of hundreds of economically important crops worldwide. The genetic structure *V. dahliae* populations is based on somatic compatibility defined by vegetative compatibility groups (VCG). Six main VCGs (1-6) have been identified so far with each of VCG1, 2, and 4 being further differentiated into subgroups A and B. Isolates within each VCG subgroup seem to be genetically similar, but suitable highly polymorphic markers are needed to further characterize genetic diversity in *V. dahliae* populations. In this study, 5 microsatellites (simple sequences repeats, SSRs) and 7 single-nucleotide polymorphic (SNP) markers were identified from a genomic DNA library highly enriched for SSRs which was constructed using the Dynabead biotin-enrichment strategy. Of the sequenced clones, 20% contained SSRs and primer pairs were designed from their flanking regions. Allelic variation on 46 *V. dahliae* isolates from diverse geographic locations, hosts and VCGs was assessed by polyacrylamide gel electrophoresis, sequence analysis of the amplified regions and fluorescence-based capillary electrophoresis. Analyses of the 5 SSR and 7 SNP markers showed a total of 50 alleles (each locus producing between 2 and 12 alleles). Phylogenetic analyses of individual and combined datasets of SSR and SNP markers showed that isolates within each VCG subgroup are molecularly similar and can be clearly differentiated from others. Markers identified in this study constitute an outstanding tool for the analysis of *V. dahliae* populations.

Evaluation of seed coating formulations of *Trichoderma harzianum* on cucumber seeds against pre- and post-emergence damping-off caused by Pythium ultimum

X. JIN (1)
(1) USDA-ARS, Mid South Area, Biological Control of Pests Research Unit, 59 Lee Road, P.O. Box 67, Stoneville, MS 38776
Phytopathology 98:S75

Seed coating formulations of *Trichoderma harzianum* were evaluated on cucumber seeds to control pre- and post-emergence damping-off caused by *Pythium ultimum* in greenhouse studies. Results showed that coating formulation H reduced the disease incidence significantly, and had the potential for commercialization.

Susceptibility of six eastern Canadian forest species to *Phytophthora ramorum*

A. Jinek (4), M. Simard (4), S. C. Brière (1), A. K. Watson (3), R. J. TWEDDELL (2), D. Rioux (4)
(1) Canadian Food Inspection Agency, Ottawa, ON, Canada; (2) Centre de recherche en horticulture, Université Laval, Québec, QC, Canada; (3) Department of Plant Science, McGill University, St-Anne-de-Bellevue, QC, Canada; (4) Natural Resources Canada, Laurentian Forestry Center, Québec, QC, Canada
Phytopathology 98:S75

Phytophthora ramorum (Pr), a recently described pathogen, causes sudden oak death, ramorum leaf blight and ramorum shoot dieback. The list of ornamental and wild plant species that are naturally infected by Pr exceeds 120 host species. Absent in the wild in eastern North America, there is concern that Pr could be introduced and spread into this area. To help assess this risk, detached leaves/needles of six eastern Canadian forest species were inoculated with Pr and the amount of necrosis and sporulation was evaluated. *Abies balsamea*, Acer *saccharum*, *Betula alleghaniensis* (Ba), *Fraxinus americana* (Fa), *Larix laricina*, and *Quercus rubra* (Qr) were the species tested whereas *Rhododendron* 'Nova Zembla' (Rh) served as positive control. With broad-leaved species (BLS), Ba and Fa were the most susceptible but sporulation was only significant on Qr, which was similar to that on Rh. Compared with the BLS, the amount of necrosis on needles was higher in both conifer species concomitantly with a higher level of sporulation. Real-time PCR results suggested that the amount of Pr DNA was higher in BLS than in conifer tissues. In addition, it clearly appeared that the young leaves of Ba, Fa and Qr were more susceptible than the older leaves.

Identification of potential plant pathogens from prematurely killed corn stalks in Wisconsin - 2007

J. C. JIRAK (1), P. D. Esker (1)
(1) University of Wisconsin, Madison, WI, USA
Phytopathology 98:S75

In the fall of 2007, numerous cornfields in southeastern Wisconsin exhibited extensive lodging due to rotten stalks, however, in a few situations, fields were found prematurely dead. Alfalfa was the previous crop in each field. Dead stalks were sampled in September to try to determine the cause of the stalk rot. Rind and pith tissues were surface disinfested and placed directly on ¼ PDA to culture possible fungal disease causing organisms. Fungi were isolated and identified based on growth and morphological characteristics. Saprophytic fungi were dominant. Putative corn pathogens, *Gibberella zeae*, *Colletotrichum graminicola*, *Bipolaris zeicola*, and *Cercospora* spp., were cultured and inoculated to corn seedlings to test Koch's postulates. *C. graminicola* and *B. zeicola* were recovered from inoculated corn seedlings. To further identify potential disease causing fungi, dead stalk and leaf tissues from field samples were ground and placed into soil as an inoculum source for corn seedlings. *C. graminicola* and *B. zeicola* were recovered using this method. To date, multiple fungal organisms have been identified, however, none appear to be the primary causal organism for the lodged corn. Research

is ongoing to link these isolations with field soil fertility and other production practices in an effort to devise an appropriate management plan.

Telia of the Asian soybean rust fungus on kudzu: Implications for overwintering in Texas
Y. JO (1), T. Isakeit (1)
(1) Texas A&M University, College Station, TX, USA
Phytopathology 98:S76

Asian soybean rust, caused by *Phakospora pachyrhizi*, is an increasingly important disease in the U.S. since its first occurrence in 2004. This disease has been detected in Texas every year since 2005, including on kudzu (*Pueraria lobata*). Overwintering survival structures of telia and teliospores were identified on kudzu leaves collected from Polk and Liberty Counties in East Texas in December 2005, December 2006 and January 2008. These two counties experienced freezing temperatures every winter. However, telia were not found on soybean leaf samples collected during the winter months from Hidalgo County in extreme southern Texas, an area of the state which has only a 50% probability of a winter freeze and did not have a winter freeze during the years of this survey. These observations indicate that soybean rust can overwinter on kudzu in an area of Texas subject to annual freezes. Continual survey on soybean and kudzu is required to estimate the significance of the telial stage on the overwinter host for the incidence of soybean rust on soybean in Texas.

Inhibition of mycelial growth of a plant pathogenic fungus by electricity
Y. JO (1), E. Kim (2), G. Jung (2), B. Kim (2)
(1) Texas A&M University, College Station, TX, USA; (2) University of Massachusetts, Amherst, MA, USA
Phytopathology 98:S76

The possibility of electricity affecting the growth of fungi has been a matter of interest for a long time, but the effects described in previous studies have not been confirmed or further utilized. In order to judge the applicability of electricity to manage plant pathogenic fungi, additional information is needed. In the current study, *Sclerotinia homoeocarpa*, causing dollar spot on various turfgrasses, was grown on the surface of potato dextrose agar medium for 6 hours. The culture was then exposed to an electric current with a constant voltage. Treatments included 1, 2, 4, 5, 6, 8, 10 and 20 V, and were applied for 0.25, 0.5, 1, 2, 3 or 4 hours. Inhibition of mycelial growth depended on the strength of the voltage and the time for which it was applied. The effective voltage inhibiting 50% of mycelial growth for one hour was between 6 and 8 V. Possible explanation of the phenomena will be discussed.

Proteins targets of ADP-ribosyltransferase type III effectors from *Pseudomonas syringae* and their effects on immune responses in plants
A. JOE (1), B. Jeong (1), Z. Fu (1), J. R. Alfano (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 98:S76

Pseudomonas syringae uses a type III secretion system (T3SS) called the Hrp T3SS to inject effectors into plant cells and suppress plant immune responses. More than 30 effectors have been identified in *P. syringae* pv. tomato DC3000. Among these, HopU1 has been recently confirmed to be an active ADP-ribosyltransferase (ADP-RT) and HopO1-1 and HopO1-2 are predicted to encode ADP-RTs. These enzymes are well known bacterial toxins in animal pathogens where they ADP-ribosylate and inhibit proteins that are involved in defense responses in animals. It has been shown that HopU1 ADP-ribosylates *Arabidopsis* RNA binding proteins including the glycine-rich RNA binding protein, AtGRP7. A *Atgrp7* mutant is more susceptible to *P. syringae* (Fu et al. 2007 Nature 447:284-288). Based on preliminary experiments AtGRP7 is appears to be involved in regulation of defense gene expression including WRKY transcription factors and pathogenesis-related (PR) genes, which can be affected by HopU1. Recombinant HopO1-1 and HopO1-2 are insoluble when expressed in *E. coli*, which has made them difficult to study. Subcloning and expression of their ADP-RT domains allowed small amounts of soluble proteins. Preliminary results suggest that HopO1-1 and HopO1-2 have weak ADP-RT activity on *Arabidopsis* protein extracts. More details of ADP-ribosylation by HopO1-1 and HopO1-2 will be presented.

The non-ribosomal peptide synthetase TxtB from plant-pathogenic *Streptomyces* uses 4-nitrotryptophan as a substrate for thaxtomin biosynthesis
E. G. JOHNSON (1), S. B. Krasnoff (5), D. R. Bignell (3), W. Chung (4), R. J. Parry (2), R. Loria (3), D. M. Gibson (5)
(1) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA; (2) Department of Chemistry, Rice University, Houston, TX, USA; (3) Department of Plant Pathology, Cornell University, Ithaca, NY, USA; (4) Seed Improvement & Propagation Station, Hsinshe Taichung, Taiwan, R.O.C; (5) USDA Agricultural Research Service, U.S. Plant, Soil, and Nutrition Laboratory, Ithaca, NY, USA
Phytopathology 98:S76

Scab-causing *Streptomyces* species require the virulence factor, thaxtomin, for disease development on economically-important root and tuber crops such as potato. Thaxtomin is a phytotoxin that functions by inhibiting cellulose synthesis in actively growing plant tissue. It consists of a cyclic dipeptide with an unusual nitrated tryptophan moiety; its biosynthesis requires two non-ribosomal peptide synthetase (NRPS) modules, TxtA and TxtB, and a complement of modifying enzymes. Although the biosynthetic enzymes are known, the order in which these enzymes act in the biosynthetic pathway has not been characterized. Analysis of a *txtAB* deletion mutant and precursor feeding experiments revealed that 4-nitrotryptophan is an intermediate in thaxtomin biosynthesis prior to assembly of the peptide backbone. This 4-nitrotryptophan moiety represents a novel substrate for NRPSs. Through identification of N-methyl-4-nitrotryptophan in a *txtA* deletion mutant and adenylation domain modeling, TxtB has been implicated as a 4-nitrotryptophan-specific NRPS module. Combining these results with our previous work on thaxtomin biosynthesis, we propose a well-supported model of the entire biosynthetic pathway.

Evidence for pathogenesis-related activity by *Erwinia amylovora* during the epiphytic phase on pear and apple flowers
K. B. JOHNSON (1), T. L. Sawyer (1), V. O. Stockwell (1), T. N. Temple (1)
(1) Oregon State University, Corvallis, OR
Phytopathology 98:S76

Prior to infection, *Erwinia amylovora* grows epiphytically on floral stigmas, which provide a conducive but nonselective habitat for bacterial growth. This nonselectivity allows for biocontrol of fire blight; although, in practice, it is very difficult to exclude *E. amylovora* completely from this habitat. We investigated the dynamics of growth suppression of *E. amylovora* by comparing the ability of virulent and avirulent strains of this pathogen to compete with each other on stigmas of pear and apple, and to compete with a co-inoculated mixture of effective bacterial antagonists. When strains were inoculated individually, virulent *E. amylovora* strain Ea153 attained the highest population size on stigmas with 'epiphytic yields' that were approximately double those of the avirulent derivative or the bacterial antagonists. In competition experiments, growth of the avirulent hrpL mutant of Ea153 was suppressed by the antagonist mixture to a greater extent than the virulent strain. Unexpectedly, the virulent strain enhanced the epiphytic yield of the antagonist mixture. Similarly, a small dose of virulent Ea153 added to inoculum of Ea153 HrpL$^-$ significantly increased the epiphytic yield of the avirulent strain. These results are consistent with the hypothesis that virulent *E. amylovora* modifies the epiphytic habitat presented by the stigma through the expression of pathogenesis-related genes, which increases resources available to itself and coincidentally to nonpathogenic competitors.

Cellulose binding domain encoding genes in Phytophthora
R. JONES (1)
(1) USDA-ARS, PSI, GIFVL, 10300 Baltimore Ave., Beltsville, MD, USA
Phytopathology 98:S76

The fungal protein binding domains that allow for adherence to cellulose are characterized by a distinct pattern of cysteines. This pattern was used to identify these regions in the genomes of *P. infestans* and *P. sojae*. Three genes (CBD1, CBD4 and CBEL) and two pseudogenes were evident in *P. infestans*, while three genes (CBD1, CBD 4 and CBEL) and one pseudogene were identified in *P. sojae*. The CBD1 gene encodes a 13 kD protein, and gene expression was found in culture and during plant infection. The CBD4 gene encodes a 12 kD protein with two cellulose binding regions. Expression was found in culture and during infection. The role of these proteins in pathogen recognition and their involvement in cell wall formation of the cellulose-containing *Phytophthora* will be presented.

Genome organization and nucleotide sequences of *Pelargonium ringspot*, *Pelargonium line pattern*, and *Elderberry latent viruses*, distinct new species within *Tombusviridae*
R. JORDAN (2), G. Kinard (1), M. Guaragna (2)
(1) National Germplasm Resources Lab, USDA-ARS, Beltsville, MD; (2) U.S. National Arboretum, Floral & Nursery Plants Research, USDA-ARS, Beltsville, MD
Phytopathology 98:S76

The complete nucleotide sequence of three isometric viruses infecting geranium have been determined. These include *Pelargonium ringspot virus* (PelRSV), *Pelargonium line pattern virus* (PLPV) and an isolate of *Elderberry latent virus* (ELV; which we have shown infects geranium and reportedly is synonymous with PelRSV). The genomic RNAs of PelRSV, PLPV, and ELV comprise 3865, 3881, and 3891 nts, respectively and each

contains six or seven open reading frames (ORFs). The arrangement of these ORFs closely resembles that of members of the genus Carmovirus in the family *Tombusviridae* and, moreover, most of the putative gene products showed high identity with proteins of this viral group. There are, however, several important distinctions. Double-stranded RNA analysis and hybridization assays demonstrate the expression of only one subgenomic RNA (sg RNA) in infected plants, whereas carmoviruses contain two sg RNAs. This single sg RNA has the potential to encode three proteins whose arrangement and coding suggest that two are expressed as a fusion protein by a -1 frameshift mechanism. The overall properties of these three viruses, along with our previously described *Pelargonium chlorotic ring pattern virus*, indicate that they are related, yet distinct from each other, and differ sufficiently from previously described Tombusviridae members to warrant assignment in a new genus provisionally designated Pelarspovirus. In addition, applying the sequence-based taxonomic conventions used for other Tombusviridae members, PelRSV and ELV should not be considered as synonymous. Results on the development of full length infectious clones will also be presented.

Genome size estimation of *Phymatotrichopsis omnivora*, the causal agent of cotton root rot

B. D. Joshi (1), C. Crane (1), S. Marek (2), I. Moncrief (2), S. MacMil (3), F. Najar (3), B. Roe (3), C. A. YOUNG (1)
(1) Noble Foundation, Ardmore, OK, USA; (2) Oklahoma State University, Stillwater, OK, USA; (3) University of Oklahoma, Norman, OK, USA
Phytopathology 98:S77

Phymatotrichopsis omnivora (Duggar) Hennebert is a destructive root pathogen of dicotyledonous plants and is endemic to Oklahoma, Texas, New Mexico and Arizona. The disease caused by this pathogen, best known as cotton root rot, has greatly hindered the production of cotton and alfalfa in this region. Currently, no practical disease management methods are available and no cotton or alfalfa varieties are known to be resistant or tolerant to this fungus. To gain insight into the genome structure and organization of this fungus, sequencing of the *P. omnivora* genome is currently underway. The draft genome sequence, obtained predominantly by '454' pyrosequencing, assembled into 75 Mb, considerably larger than initially expected. However, this large assembly could result from the multinucleate, heterokaryotic nature of this presumably asexual fungus. To support sequencing efforts, we are determining the genome size of *P. omnivora* using electrophoretic chromosome separations and size estimation based on a BAC library. At least seven chromosomal DNA bands, ranging in size from 3.8 to ~10 Mb and totaling ~40 Mb, were observed by pulse-field gel electrophoresis. The chromosome number was also determined by hybridization of *P. omnivora* DNA with a telomere probe. Probes detecting single copy genes, such as beta tubulin and RNA polymerase II subunit 2, were hybridized against an arrayed *P. omnivora* BAC library that was estimated to represent 10x genome coverage based on a 40 Mb genome size. The number of hybridizing clones represented by these genes was less than expected, inferring a genome size much larger than 40 Mb. The genome sequence of *P. omnivora* should enhance our understanding of its intriguing biology and should help in the development of strategies to control cotton root rot.

Isolation, purification and biochemical characterization of a polygalacturonase produced by *Penicillium solitum* in 'Golden Delicious' apple (*Malus domestica* Borkh)

W. M. JURICK, II (3), I. Vico (2), J. L. McEvoy (3), W. Janisiewicz (1), W. S. Conway (3)
(1) Appalachian Fruit Research Station, ARS-USDA, Kearneysville, WV; (2) Institute for Plant and Food Protection, Faculty of Agriculture, University of Belgrade, Serbia; (3) Produce Quality and Safety Lab, ARS-USDA, Beltsville, MD
Phytopathology 98:S77

Penicillum solitum and *P. expansum* are filamentous fungal pathogens that cause blue mold of apples resulting in significant economic losses postharvest. However, *P. solitum* is significantly less virulent than *P. expansum* and the biochemical basis for host invasion by *P. solitum* is unknown. Generally, necrotrophic fungi produce an array of enzymes to break down cell walls and gain ingress into the host. However, previous investigations have demonstrated that a polygalacturonase (PG) produced by *P. expansum* is involved in tissue maceration and apple fruit colonization. Therefore, this work focuses on the isolation, purification, and characterization of PG from *P. solitum* produced during apple fruit infection. The pathogen-infected apple tissue was excised, filtered through cheesecloth, and treated with step-wise additions of ammonium sulfate to reach 90% saturation. The 90% ammonium sulfate cut was fractionated on a Sephacryl S-200 gel filtration column, further purified on a CM-Sephadex cation exchange column, and eluted using a linear salt gradient. Fractions exhibiting PG activity were identified and used for preliminary biochemical characterization. Optimum PG activity occurred between pH 4.5 - 5.0, and was nearly undetectable after boiling (100°C) for 5 minutes. Additional biochemical analyses of the PG produced by *P. solitum* will be compared to the PG produced by *P. expansum* in decayed apple fruit. Results from this study will provide insight into the physiological basis for the observed differences in virulence between *P. solitum* and *P. expansum*.

Bitternut hickory stem cankers and bark necrosis resulting from inoculations with *Ceratocystis* spp. and *Fusarium solani*

J. JUZWIK (3), L. Haugen (2), J. Park (1)
(1) Dept. of Plant Pathology, University of Minnesota, St. Paul, MN, USA; (2) NA State & Private Forestry, U.S. Forest Service, St. Paul, MN, USA; (3) Northern Research Station, U.S. Forest Service, St. Paul, MN, USA
Phytopathology 98:S77

Higher than expected levels of decline and mortality of bitternut hickory (*Carya cordiformis*) have recently been observed in states of the North Central and Northeastern U.S. During 2006–07 surveys of declining hickory stands in Iowa, Minnesota, and Wisconsin, we frequently isolated *Ceratocystis smalleyi* from diffuse cankers and *Fusarium solani* from sunken, annual cankers. To determine the potential role of putative canker fungi in hickory decline, we inoculated three offset points on stems of healthy bitternut hickory (13 to 30 cm diameter) with one of two isolates each of *C. smalleyi*, *C. caryae* (a sister species), and *F. solani*. Within 6 months we found differing levels of canker occurrence and inner bark necrosis. Canker incidence (9 of 12 stems) and inner bark necrosis (average 17 cm^2 for 11 stems) were highest for *C. smalleyi* isolates. Similar canker incidences (2 of 12 and 1 of 12 stems) and necrotic bark areas (average 4 cm^2 for 11 and 5 cm^2 for 12 stems) were found for *C. caryae* and *F. solani*, respectively. No cankers resulted from control inoculations and minimal bark necrosis (average 2 cm^2) developed. Early harvest of one replicate found discolored sapwood associated with stem cankers; the inoculated fungi were recovered from the wood. Based on preliminary results, *C. smalleyi* is the species most likely to contribute to bitternut decline and death.

Preventing spread of the oak wilt pathogen in an operational disease control program

J. Juzwik (4), J. O'BRIEN (2), C. Evenson (5), P. Castillo (3), G. Mahal (1)
(1) Dept. of Forest Resources, University of Minnesota, St. Paul, MN; (2) NA State & Private Forestry, U.S. Forest Service, St. Paul, MN; (3) Northern Research Station, U.S. Forest Service, Mt. Pleasant, MI, USA; (4) Northern Research Station, U.S. Forest Service, St. Paul, MN, USA; (5) Three Rivers Park District, Plymouth, MN, USA
Phytopathology 98:S77

The vibratory plow is the primary tool used to prevent root graft spread of the oak wilt (OW) pathogen, *Ceratocystis fagacearum*, in Minnesota. Different options exist for eliminating sources (primarily red oaks species, *Quercus* section *Lobatae*) of fungal spore mats, thus reducing the risk of overland spread. Very few data, however, document the efficacy and/or impacts of these methods in organized OW control programs. Annual stem maps were created in a geographical information system to depict all healthy and wilted oaks in 25 active OW centers in forested park land from the time of plow treatments (1997–99) through 2005. At four sites, treatments failed to prevent root graft spread outside the primary line between 4 and 6 years after plowing. Annual removal of wilted red oaks from these sites (69, based on stem maps) was the selected, but not fully implemented, option for controlling overland spread. In comparison, approximately 279 red oaks (from 2 to 24 per site) would have been removed at the time of plowing if a "cut to the line" option had been used. Results show plow lines are >80% effective in preventing root graft spread for at least 5 years. Removal of likely OW spore producing trees would reduce incidence of new pathogen introductions, affording even greater control. Further comparison of the costs and benefits for the red oak removal options is needed.

Invasion and management of coffee leaf rust in high altitude coffee plantations in Kenya

G. KAIRU (1)
(1) Coffee Research Foundation, Ruiru, Kenya
Phytopathology 98:S77

Coffee leaf rust (*Hemileia vastatrix* Berk. & Br.) is an important disease of coffee (*Coffea arabica* L.) grown in Kenya. Its occurrence was first reported in 1913. In the past, high incidences of coffee leaf rust were associated with the lower, warmer agro-eco zones up to about 1800 m. This position has now changed. Seasonal incidence of coffee leaf rust recorded from October 2007 to February 2008 in four sites located at different altitudes (1570 m to 1950 m) has indicated escalation of coffee leaf rust in higher altitude plantations where the disease was not a problem in the past. Coffee leaf rust prefers wet, warm weather. Uredospores of *H. vastatrix* require a water film for 1–3 hrs at 20–25°C, optimum 23°C to germinate and form appressoria usually in darkness. It is therefore likely that the frequency of these critical requirements is

increasing in the higher altitude plantations which were previously cooler and unfavourable to the development of coffee leaf rust. Sprays of chlorothalonil alone or as a proprietary mixture with azoxystrobin resulted in 83% and 86% reduction of coffee leaf rust respectively. These sprays were initially designed to target coffee berry disease (*Colletotrichum kahawae* Waller & Bridge) which is the more important problem in the higher agro-eco zone plantations. Azoxystrobin is a recommended fungicide for management of coffee leaf rust but not chlorothalonil. The observed efficacy of chlorothalonil against coffee leaf rust is a new record. The implications of these findings in managing a progressive invasion of coffee leaf rust in the higher altitude but gradually warming up plantations will be discussed.

Clavibacter michiganensis subsp. _michiganensis_ threshold levels required for transmission by naturally-infested tomato seed

W. S. KANESHIRO (2), D. M. Ingram (1), A. M. Alvarez (2)
(1) Central Mississippi Research and Extension Center, 1320 Seven Springs Road, Raymond, MS 39154; (2) University of Hawaii, Dept. of Plant & Environmental Protection Sciences, 3190 Maile Way, St. John 307, Honolulu, HI 96822
Phytopathology 98:S78

Tomato seeds infested with the bacterial canker pathogen *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) can produce infected plants (seed transmission) but the required threshold population per seed is unknown. Initial populations of *Cmm* resulting in reduction of germination and subsequent plant infection were investigated. Seeds harvested from *Cmm*-infected tomatoes were germinated and emergent seedlings transplanted and grown to maturity. To establish pathogen threshold values, the population distribution of *Cmm* per seed was determined at experiment initiation. Simultaneous experiments in Hawaii and Mississippi were conducted to compare disease development between sites. Germination of *Cmm*-infested seed varied greatly depending on the sowing method used and typically ranged between 75% and 100%. Symptom development and overall disease severity differed between growing sites. In Hawaii, infected plants exhibited symptoms in the seedling and early transplant stage whereas Mississippi plants expressed symptoms closer to flowering and fruit set. About 40% of seeds that germinated on blotters in Hawaii developed symptoms within two weeks post-emergence. In Mississippi, seedlings were asymptomatic but up to 37% of plants developed symptoms by harvest. The disease occurred at both sites when *Cmm* populations were approximately 10^2 CFU per seed, indicating that this is the probable threshold value for transmission of *Cmm* on naturally-infested seeds.

Search of plant products to control some fungal pathogens

B. KANG (1), S. Kim (1), D. Kim (1), K. Choi (1)
(1) Jeollanamdo Agricultural Research and Extension Services, Naju-si, Jeollanam-do, Republic of Korea
Phytopathology 98:S78

In order to search potent antifungal substances from domestic plants, 30 plants cultivated in Korea were collected. More than 30 plant species tested for their antifungal effects on radial growth *Pyricularia grisea* causing rice blast, *Rhizoctonia solani* causing rice sheath blight, *Phytophthora* sp. causing phytophthora root rot and *Pythium* sp. causing pythium root rot of cultivated strawberry. In this experiment, MeOH and EtOH extracts of effectively inhibited the radial growth and spore germination of these fungi by using filter paper and poisoned food methods. *Glycyrrhiza uralensis*, *Anemarrhena asphodeloides*, *Coptis chinensis* and gallnut extracts effectively inhibited the growth of rice blast with filter paper disc method. The extracts of *C. chinensis* that showed potent antifungal activity reduced the occurrence of leaf blast when applied in the field. Especially, barks of *Albizzia julibrissin* showed potent antifungal activities against *P. grisea* and *R. solani*. The *in vivo* tests indicated that *Acorus gramineus*, *C. chinensis* extracts effectively reduce disease incidence of root rot for 72 hours after inoculation. These results suggest that the antifungal activity of some plant extracts substantially inhibited the growth of 4 plant disease tested.

Development of beneficial microorganism for biology control of tomato Fusarium wilt

B. KANG (2), D. Kim (2), S. Kim (2), K. Choi (2), J. Lee (1), Y. Kim (1)
(1) Chonnam National University, Gwangju, Republic of Korea; (2) Jeollanamdo Agricultural Research and Extension Services, Naju-si, Jeollanam-do, Republic of Korea
Phytopathology 98:S78

In order to select effective antagonists to control Fusarium wilt of tomato, 345 strains were isolated from rhizosphere and suppressive soil, etc. in Korea. Among these microbes, a bacterial strain JN134 showed most effective inhibition against mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*. Based on 16S ribosomal DNA sequence analysis, the bacterial strain identified as *Pseudomonas* sp. The bacterium also suppressed mycelial growth *in vivo* of other plant fungal pathogens: *Botrytis cinerea*, *Colletotrichum acutatum*, *Rhizoctonia solani*, *Phytophthora infestans*, and *Pyricularia grisea*. JN134 was an effective root colonizer of tomato plants being recovered at a level of more than 1×10^8 cfu/g root even 18 days after inoculation. Application of JN134 by root drenching significantly suppressed disease incidence of tomato Fusarium wilt. In greenhouse test, the cell suspension(10^8/g) of the isolate JN134 was more effective to control the disease occurrence than the suspension at lower concentrations. Root colonization of JN134 showed positive effect on the growth of the tomato. These results indicated that JN134 has potential biological control agent.

Two viruses are associated with Carnation necrotic fleck disease

A. V. KARASEV (3), V. V. Dolja (1), M. A. Guaragna (2), R. Jordan (2)
(1) Oregon State University; (2) USDA-ARS; (3) University of Idaho
Phytopathology 98:S78

While analyzing virus-specific RNAs from plants infected with *Carnation necrotic fleck virus* (CNFV), we found that infected plants contained two distinct virus species of the same genus *Closterovirus*. One closterovirus genome, which we propose to name *Carnation yellow fleck virus*, CYFV, was sequenced in its entirety, and about 70% of the second closterovirus genome, which we propose to name CNFV was sequenced as well. Phylogenetic analyses of proteins that are conserved throughout the family *Closteroviridae* demonstrated that both viruses belong to a lineage of aphid-transmitted closteroviruses typified by *Beet yellows virus*. The two viruses can be detected and distinguished in RT-PCR using total RNA isolated from carnation tissues. The two viruses could also be distinguished using several CNFV-specific MAbs and a polyclonal antiserum produced to a bacterially expressed CYFV capsid protein. Single and mixed infections were identified in a series of screened carnation plants.

The _Pseudomonas syringae_ HrpJ protein is type III secreted, required for plant pathogenesis, and controls the secretion of accessory proteins

A. D. KARPISEK (1), Z. Fu (1), J. R. Alfano (1)
(1) University of Nebraska - Lincoln, NE, USA
Phytopathology 98:S78

Pseudomanas syringae is a bacterial plant pathogen that requires a type III secretion system (T3SS) for pathogenicity. The T3SS is encoded by genes found in the *hrc/hrp* cluster. One gene in the *hrc/hrp* cluster, *hrpJ* encodes a protein that shares similarity to YopN, a protein secreted by *Yersinia* T3SS. A *hrpJ* nonpolar mutant shows reduction in its ability to cause disease in host plants and a hypersensitive response (HR) on resistant plants. This indicates that HrpJ is an essential protein for an operational T3SS. Even though HrpJ is required by the bacterium for wild type levels of pathogenicity, the reduction in virulence is not due directly to the absence of HrpJ, but rather its inability to secrete accessory proteins that aid in the translocation of effector proteins into the host cell. These accessory proteins HrpZ, HrpW and HrpK are not secreted or their secretion is greatly reduced from a *hrpJ* mutant. Interestingly, HrpJ itself is shown to be secreted, but secretion deficient derivatives of HrpJ are able to restore secretion of HrpZ, a member of the harpin protein class. Harpins are predicted to modify the cell wall and/or acts as translocators by forming pores in the plant plasma membrane. Additionally, a mutant derivative of HrpZ lacking a C-terminal portion regains the ability to be secreted in a *hrpJ* mutant. This suggests that a domain resides in this portion of HrpZ that might interact with HrpJ to be secretion competent, and when removed the HrpZ protein is no longer subject to control by HrpJ. Thus, HrpJ appears to act as a control protein that is required for a specific set of proteins to be secreted by the *P. syringae* T3SS. A model predicting how HrpJ exerts its control on the flow of proteins through the T3SS apparatus will be presented.

Analysis of molecular variability among the isolates of _Verticillium dahliae_ from diverse host species based on fluorescence-based amplified fragment length polymorphism

M. KARUNAKARAN (2), G. E. Vallad (3), Z. K. Atallah (2), S. J. Klosterman (1), M. R. Davis (2), K. V. Subbarao (2)
(1) USDA-ARS, Salinas, CA, USA; (2) University of California, Davis, CA, USA; (3) University of Florida, Wimauma, FL, USA
Phytopathology 98:S78

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae*, poses a serious threat to economically important crops including the $1.5 billion California lettuce industry. Development of wilt resistant cultivars for commercial deployment offers the most practical strategy for disease control, which relies on thorough characterizations of pathogen populations since at least two races of V. dahliae infect lettuce and various isolates have different host specificities. Fluorescence-based amplified fragment length polymorphism (fbAFLP) was used to identify the phylogenetic relationships at the intraspecific level in *V. dahliae*. Molecular diversity of ~300 isolates, recovered from different crop species was characterized by fbAFLP analysis and pathogenicity assay. Fifteen primer combinations gave ~400 scorable frag-

ments. Unambiguous peaks within the range of 50-500 bp were selected and used to generate matrices of genetic distance among isolates for cluster analysis and for analysis of molecular variance. To identify the races among the isolates used in the AFLP analyses, differential cultivars La Brillante (resistant) and Salinas (susceptible) were inoculated by soil drenching and evaluated for wilt severity 13 wks post-inoculation. Preliminary analysis of the data suggests that 15–18% of the isolates belonged to race 2 and the rest were race 1. AFLP analysis did not reveal either host specific or race-specific groupings.

Defective cellulase production of *Xanthomonas axonopodis* pv. *glycines* ppsA mutant strain triggered systemic resistance to soybean bacterial pustule

S. KASEM (1), S. Prathuangwong (1)
(1) Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
Phytopathology 98:S79

Xanthomonas axonopodis pv. *glycines* (Xag) mutant strain KUMNTP2 obtained from Tn5 mutagenesis in phosphoenal pyruvatesyntase (ppsA) genes showed deficient in disease symptom and HR development on susceptible soybean and tobacco respectively. Mutant gave alteration in the level of virulent factor affected to its cellulase production. This experiment revealed a defective cellulase and pathogenicity mutant of Xag had ability to trigger systemic resistance induction of soybean against virulent Xag wildtype infection. Different soybean leaf treatments included KUMNTP2, wildtype and complement, and ddH2O inoculants were sampled at different time intervals for defense related enzyme analysis. Soybean leaf treatments with wildtype and mutant induced expression of SAR-related genes which the 4 defense related enzymes were detected at 1 day after pathogen inoculation. Coinoculation of mutant pretreated-plants induced significantly greater amount of beta-1,3 glucanase and phenylalanine ammonia lyases (PAL) at 3 days of challenged inoculation. The activity of phenolic compounds and peroxidase (POX) detection exhibited at equivalent levels of all treatments investigated. The beta-1,3 glucanase and PAL were found to accumulate in non-pathogenic mutant leaf tissues at 1 day after pathogen challenged and reached maximum at 3rd and 4th day respectively. In pathogen inoculated plants, the accumulation of beta-1,3 glucanase and PAL started at the 2nd and reached maximum at 3rd and 4th then, drastically decreased at 4th and 5th day after the pathogen inoculation respectively. Pathogen population was significantly smaller in mutant pretreated-plants after 4 days of wildtype inoculation, while the pathogen treated alone remained rapid growth than single mutant treated plants, suggesting that pathogen growth was mediated by those enzyme defense responses. This work has generated an interest in resistance strategies exploiting weakly or non-pathogenic strains as a tool for appropriate innate defense mechanism in plant breeding program, and might be the model contribution to development of novel strategies for disease management.

Characterization of population dynamics and diversity of *Ralstonia solanacearum* populations isolated from flue-cured tobacco in North Carolina

M. L. KATAWCZIK (1), A. Mila (1)
(1) North Carolina State University, Raleigh, NC, USA
Phytopathology 98:S79

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is a serious vascular disease on members of the Solanaceae family including tobacco. In 2007 in North Carolina a total of six naturally infected fields with BW were sampled. On two of these fields, there were on-farm trials, where 30 flue-cured tobacco varieties were planted, and on four farms either K 326 or K 346 planted. Fifty-two isolates of *Ralstonia solanacearum* were obtained from flue-cured tobacco varieties K 326, K 346, K 394, and Sp168. From the four varieties, two (K 326 and K 394) have low whereas K 346 and Sp 168 have moderate to high resistance to BW. These varieties were chosen to determine if a certain variety selected for isolates of *R. solanacearum* with different levels of aggressiveness or pathogenicity. The sampling method also allowed for comparisons of isolates from the same variety between fields to investigate diversity among locations. Population dynamics and diversity were assessed by comparing race, biovar, pathogenicity, hypersensitivity response and rep-PCR fingerprints using BOX, REP, and ERIC primers. Information from this study will be used as a base to compare the diversity of *R. solanacearum* populations across North Carolina and to understand the variability within a single field when different varieties or a single variety are used.

Novel reporter based constructs to study the evolution rate of LRR regions of plant resistance genes

P. KATHIRIA (1), Y. Yao (1), I. Kovalchuk (1)
(1) University of Lethbridge, Lethbridge, Canada
Phytopathology 98:S79

The interaction between Tobacco mosaic virus (TMV) and *Nicotiana tabacum* is one of the widely studied models of viral pathogenesis. Previously, experiments with this model revealed a novel systemic signal during compatible plant pathogen interactions. The signal travels faster than the virus in the infected plants. It leads to higher somatic and meiotic recombination events in the infected and subsequent non-infected generations. Additionally, the plants which receive the signal exhibit epigenetic changes and altered onset of virus infection symptoms. Also, the Leucine rich repeats (LRR) regions of the plant genome are significantly hypo-methylated and show higher rate of rearrangements in infected plants. These evidences indicate a possibility of higher rate of recombination events in the LRR regions of plant genome after perception of the signal. Higher rate of recombination in LRR region can increase the possibility of creation of new recombinant LRR regions which might be able to recognize the previously compatible pathogen. To investigate the possibility of higher recombination in LRR regions post virus infection, novel recombination based reporter constructs were designed. These constructs lead to reporter gene expression on occurrence of a recombination event in a specific sequence of interest. Such constructs also gives us the possibility of easier cloning and sequence analysis of such recombination events. Studies using these constructs have a potential to overcome inherent problems with conventional approaches and provide us better understanding of such phenomena.

Effects of Tween 20 on wheat seedling leaf infection by *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein.) Petch.)

Y. Kawasaki (1), J. Y. Takemoto (1), C. K. EVANS (1)
(1) Utah State University, Biology Department, Logan, UT 84322
Phytopathology 98:S79

Tween 20 (T20), a surfactant that lowers H_2O surface tension, facilitates the adhesion of inoculum to hydrophobic wheat leaf surfaces. Some surfactants are phytotoxic in some plants. Four experiments were done to measure the phytotoxicity of T20 concentration on wheat leaves and how the surfactant influences infection by *F. graminearum* (*F.g.*). The inocula (10-microliter/leaf, 10^5-macroconidia/ml) were prepared in 0.2, 0.8, and 2.0-ml/l of T20 solutions containing 2.5-g/l agar in distilled H_2O (WA). Those inocula were applied on primary leaves of Alsen (AL) and Frontana (FR) wheat cultivars 14 days after seeding. Inoculated plants were incubated in a dew chamber (23°C, 12/12 h:light/dark, 100% humidity) for 72 hrs and then placed under lights on a lab bench. After 24 h, percent lesion area (%LA) and relative chlorophyll content (RCC) were measured using digital image and spectral absorbance analyses, respectively. The leaves of AL inoculated with *F.g.* in WA with 2.0-ml/l T20 had significantly ($P = 0.05$) greater %LA (6.39%) and reduced RCC (5.28) compared to leaves of FR (0.06%LA, 15.62 RCC), respectively. There were no significant differences ($P = 0.05$) in the results among any treatments on the FR cultivar's leaves. Control treatments of AL and FR cultivars were not significantly different ($P = 0.05$) compared to the non-inoculated control treatment of each cultivar, respectively. T20 did not cause significant phytotoxicity on either wheat cultivar. The data show that AL and FR cultivars have significantly differing leaf infection reactions when inoculated with *F.g.* This technique can be used for investigations of experimental compounds to evaluate their potential to inhibit wheat leaf infection by *F.g.* using AL and FR.

Population genetic data analysis of *Tomato spotted wilt virus* on peanut in North Carolina and Virginia

A. C. KAYE (1), G. G. Kennedy (1), B. B. Shew (1), M. A. Cubeta (1), J. W. Moyer (1)
(1) North Carolina State University
Phytopathology 98:S79

Tomato spotted wilt virus (TSWV) is an important pathogen of peanut worldwide and can cause severe yield loss and plant death. In 2007, 180 leaf samples were collected from peanuts exhibiting symptoms of TSWV from fields in North Carolina and Virginia. One hundred thirty-five samples tested positive for TSWV with ELISA and were used for extraction of RNA and cDNA synthesis. Primers specific to three optimal regions (RdRp, 1000 nt; M, 699 nt; N, 720 nt) of the TSWV genome were used to amplify and sequence cDNA from each sample. Sequence data from 2007 were combined with sequence data from TSWV-infected peanuts sampled in 2005 and 2006 and subjected to phylogenetic analysis using the Suite of Nucleotide Analysis Programs. Application of these programs identified more than 100 haplotypes and segregating nucleotide sites for each genomic region, indicating high levels of genetic diversity in the populations. Noncontiguous, non-recombining regions of sequence were distilled from the larger sequence data set for use in calculation of population parameter inferences. These regions show that populations of TSWV are subdivided by geographical area and disease symptomatology when applying Hudson's Nearest Neighbor statistic Snn. Tests of neutrality (Tajima's *D*, Fu and Li's *D**, Fu and Li's *F**, Fu's *Fs*) indicate significant selection pressure on the virus population. Future analysis

will apply the coalescent process to infer evolutionary relationships of the sampled isolates.

Effect of nozzle type and water volume on dollar spot control in creeping bentgrass

M. M. KENNELLY (1), R. Wolf (1)
(1) Kansas State University, Manhattan, KS, USA
Phytopathology 98:S80

A field study was conducted in Aug-Sep 2007 to determine the influence of nozzle type and water volume on the control of dollar spot, caused by *Sclerotinia homoeocarpa*. Plots were 1.5 × 1.5 m and were arranged in a randomized complete block design with four replications. Chlorothalonil (4.5 kg ai/ha) was applied on a 14-d interval with the following treatment variations. The nozzle types included were: XR TeeJet, TurfJet, Air Induction TeeJet, Turbo TwinJet, and Turbo Drop Twin Fan. Pressure was kept constant at 276 kPa, and for each nozzle type, three flow rates (ex: XR11002, XR11004, and XR11008) were used to produce water volumes equivalent to 206, 412, or 824 liters per/ha. Disease was assessed every 10–20 days by counting the number of dollar spot infection centers in a 0.4 × 0.4 m square in the center of each plot where coverage was complete. On two rating dates there were no significant differences among any treatments. On the third rating date, all nozzle-volume combinations reduced disease significantly compared to the untreated controls except for the TurfJet nozzles at the lower water volumes (TJ02 and TJ04). There were no significant differences among water rates for any other nozzles. These results show that nozzle selection can affect dollar spot development, and some nozzles can be paired with low water rates without compromising disease control.

The effects of temperature and leaf wetness duration on the development of gray leaf spot in kikuyugrass turf

J. KERN (1), W. Uddin (1), F. Wong (2)
(1) The Pennsylvania State University; (2) University of California-Riverside
Phytopathology 98:S80

Gray leaf spot, caused by *Magnaporthe oryzae*, is a newly emerging disease of kikuyugrass turf. Effects of environmental factors on development of gray leaf spot in kikuyugrass turf are virtually unknown. This study investigated the effects of temperature and leaf wetness duration on the development of gray leaf spot in kikuyugrass. The experiment was a split-plot design where temperature (20, 24, 28, and 32°C) was the main-plot factor and leaf wetness duration (LWD; 3 to 24 h at 3 h intervals) was sub-plot factor. There were six replications of treatments. Six-week-old kikuyugrass plants were inoculated with *M. oryzae* (5×10^4 conidia/ml water), and the plants were placed in four controlled environment chambers at each temperature. The experiment was conducted four times with each chamber exposed to a different temperature treatment in each run of the experiment. Development of gray leaf spot was monitored daily and incubation periods were recorded. Disease incidence and severity were assessed seven days after inoculation. There were significant affects of temperature and LWD on disease incidence and severity. Additionally, there were significant interactions between temperature and LWD. Gray leaf spot incidence and severity increased with increasing LWD at all four temperatures tested, and they were greatest at 24°C and 24 h LWD. Results from this study will be used in development of a predictive model for gray leaf spot of kikuyugrass turf.

Impact of temperature on virulence of *Pythium volutum* toward creeping bentgrass

J. P. KERNS (2), L. P. Tredway (1)
(1) NCSU, Dept. of Plant Pathology; (2) University of Wisconsin-Madison, Dept. of Plant Pathology
Phytopathology 98:S80

Symptoms of Pythium root dysfunction (PRD) in creeping bentgrass are most common in the summer during periods of heat and drought stress. However, our observations indicate that *Pythium volutum*, a causal agent of PRD, is most active during the fall and spring. A mycelial growth assay was performed by incubating 11 *P. volutum* isolates at 10C, 12C, 14C, 18C, 22C, 24C, 26C, 28C, and 31C. To determine the optimal temperature range for infection by *P. volutum*, mature 'A-1' creeping bentgrass plants were inoculated with one of 5 *P. volutum* isolates. Inoculated plants were transferred to growth chambers at constant 12C, 16C, 20C, 24C, 28C or 32C (12 hr day/night cycles), then the temperature in all chambers was increased to 32C/26C day/night to induce foliar symptoms. Growth assays demonstrated that *P. volutum* grows best when temperatures are between 18C and 26C. Typical PRD foliar symptoms developed in the 12C, 16C, 20C and 24C treatments two weeks after the temperature in all growth chambers was elevated to 32C/26C day/night. Severity of PRD was greatest when *P. volutum* infects creeping bentgrass roots at 16C. Reductions in root depth and/or root mass were observed prior to raising the temperature to 32C/26C in the 12C, 16C, and 20C temperature treatments. Once the temperature was elevated, extensive root dieback occurred at 12C, 16C, 20C, and 24C. These results demonstrate that *P. volutum* is most active at temperatures prevalent during the fall and spring in NC, supporting our hypothesis that the majority of root infection occurs during this time.

Evaluating strategies for managing Cercospora leaf spot of sugarbeet

M. F. KHAN (1)
(1) Plant Pathology Department, North Dakota State University & University of Minnesota, Fargo, ND, USA
Phytopathology 98:S80

Cercospora leaf spot, caused by *Cercospora beticola*, is the most damaging foliar disease of sugarbeet in North Dakota and Minnesota. Different disease management strategies were evaluated in 2006 and 2007 at Foxhome, MN. Fungicides were applied when required in an alternation program starting at initial symptoms. Plants were defoliated in July in 2006, and July and August in 2007, to avoid the disease. There were also untreated check plots. Disease severity level was high in the untreated check in both years. The use of fungicides consistently provided acceptable disease control and resulted in the highest recoverable sucrose because of higher root yields with significantly higher sucrose concentrations. Defoliated plants had the least amount of Cercospora leaf spot. However, defoliation adversely impacted the photosynthetic capacity of the plant resulting in reduced yield. In 2006, defoliation resulted in similar root yield and recoverable sucrose as the untreated check. However, in 2007, defoliation resulted in lower root yield, sucrose concentration and recoverable sucrose than the untreated check. Lower yield of defoliated plots in 2007 was probably a result of slow re-growth of leaves because there was 45% less rainfall in July through September in 2007 compared to 2006. The use of defoliation as an avoidance strategy for managing Cercospora leaf spot of sugarbeet and producing high tonnage roots with high sugar concentrations was not effective.

Blast Interfacial Complex, a novel in planta structure that accumulates effector proteins of rice blast fungus *Magnaporthe oryzae*

C. KHANG (3), R. Berruyer (5), S. Park (1), P. Kankanala (3), K. Czymmek (2), S. Kang (4), B. Valent (3)
(1) Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, Seoul National University, Seoul 151-921, Korea; (2) Department of Biological Sciences, University of Delaware, Newark, DE, USA; (3) Department of Plant Pathology, Kansas State University, Manhattan, KS; (4) Department of Plant Pathology, The Pennsylvania State University, University Park, PA; (5) UMR BGPI, AGRO.M-CIRAD-INRA, Montpellier, France
Phytopathology 98:S80

The hemibiotrophic fungus *Magnaporthe oryzae* causes the devastating rice blast disease using specialized intracellular invasive hyphae (IH) that successively invade living plant cells. IH differentiate from thin filamentous primary hyphae that form immediately after the appressorial penetration peg breaches the exterior plant surface. At this point, IH become enclosed in a generally tight-fitting, plant-derived Extra-Invasive Hyphal Membrane (EIHM). Blast effector proteins are secreted from IH to manipulate host responses, but the route of their delivery across the EIHM to reach the host cytoplasm is not understood. Here we show that fusion proteins between blast effectors, AVR-Pita and PWL, and enhanced green fluorescent protein (EGFP) are secreted by the fungus into a previously unrecognized structure, the Blast Interfacial Complex (BIC). Live cell imaging showed that fusion proteins first appeared in primary BICs at the growing tips of primary hyphae, in the previously reported EIHM caps. At the point where primary hyphae differentiated into bulbous IH, the fluorescent BICs moved to the sides of enlarging IH and remained fluorescent at these same locations as long as IH grew within the cell. When the fungus moved into neighboring cells, fluorescence accumulated in secondary BICs at the tips of filamentous IH that grew immediately after crossing the plant cell wall. Again, the fluorescent tip structures moved to the side when the thin hyphae thickened into IH. BICs reside between the fungal cell wall and the EIHM in dynamic association with plant cytoplasm as IH begin to grow. Correlative light and electron microscopy showed that BICs contained complex lamellar membranes and vesicles. BIC localization requires the N-terminal signal peptide of these effector proteins. We hypothesize that BICs are involved in delivering fungal effectors proteins inside plant cells.

Microbial antagonists of *Verticillium dahliae* colonize cotton root system

S. M. KHODJIBAEVA (1), G. D. Zolotilina (1), J. J. Tashpulatov (1), R. Stipanovic (2)
(1) Institute of Microbiology, 7B, A.Kadyri street, Tashkent, Uzbekistan, 100128; (2) USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX, USA
Phytopathology 98:S80

Verticillium wilt remains one of the most severe diseases affecting cotton production in Uzbekistan. We are investigating microbial antagonist to control

this pathogen. To this end, we have identified several antagonists of *Verticillium dahliae* (*Bacillus* sp. 234, *Bacillus* sp. 3, *Streptomyces roseoflavus* 33, *Streptomyces* sp. 7, *Stachybotrys chartarum* 295 and *Trichoderma viride* 445) that colonize cotton roots. Studies conducted on cotton resistant variety Bukhara-6 under sterile and non-sterile conditions revealed that in addition to suppressing pathogen activity, selected antagonists also stimulated seed germination (15%) and growth and development of cotton seedlings (50–100%). Studies conducted under sterile conditions revealed that cotton seeds treated with the selected antagonists effectively colonized cotton roots. Under non-sterile conditions, the selected antagonists colonized cotton roots and compete with indigenous microorganisms. Simultaneously, antagonists protected cotton plants from infection by *V. dahliae*, but did not greatly affect useful soil microflora. Thus, the selected microbial agents provided reliable biocontrol activity against *V. dahliae*. The selected antagonists possess certain biotechnological value and may be used for development of new microbiological means for plant protection.
This work was conducted within STCU project P-226 "Biological control of Verticillium wilt in cotton".

Role of fimbrial and afimbrial adhesins and gum production on *Xylella fastidiosa* insect transmission
N. KILLINY (1), R. Almeida (1)
(1) Department of Environment Science, Policy and Management, University of California, Berkeley, USA
Phytopathology 98:S81

The xylem-limited bacterial pathogen *Xylella fastidiosa* causes many plant diseases. This bacterium attaches to, multiplies in, and is transmitted from the foregut of sharpshooter leafhoppers. We conducted *in vitro* attachment assays and showed that *X. fastidiosa* cells attach to carbohydrates. Cells treated with proteases showed less attachment. Reduced attachment in the presence of certain sugars suggested that binding to the foregut of vectors is carbohydrate mediated and that proteins on *X. fastidiosa* cell membrane function as lectins. Among numerous *X. fastidiosa* knockout mutants tested, only mutants of hemagglutinin-like proteins were affected in their binding to leafhopper foregut extracts and polysaccharides. Mutants of hemagglutinin-like proteins also have reduced transmission rates. Fimbrial adhesins seem not to be required for *X. fastidiosa* transmission, while gum mutants were found to be non-transmissible. Accordingly, we suggest a hypothesis describing the complex interactions between *X. fastidiosa* and its vectors' foregut surface. The initial *X. fastidiosa*-vector interaction is mediated by carbohydrate binding proteins, including hemaglutinin-like proteins. In this step cells attach sideways to the cuticle in the foregut of insects. In the second stage cells attach polarly and nonspecifically to the foregut surface via the fimbrial adhesins. Cells multiply and form a mature biofilm. We hypothesize that exopolysaccharides produced by *X. fastidiosa* cells assure initial attachment and maintenance of the biofilm. *X. fastidiosa* colonization of vectors seems to be a stepwise process, much like the formation of a biofilm on solid surfaces.

Galactinol is a signaling component of the induced systemic resistance caused by *Pseudomonas chlororaphis* O6 root colonization
M. Kim (3), S. Cho (3), Y. Im (3), H. Hoon (2), Y. Kim (3), C. Ryu (1), K. Yang (3), G. Chung (3), B. CHO (3)
(1) Systems Microbiology Research Center, KRIBB, Daejon 305-333, South Korea; (2) Department of Agricultural Biochemistry, College of Agri. and Life Sci. Chonnam National University, Gwangju, South Korea; (3) Department of Plant Biotechnology, College of Agri. and Life Sci., Chonnam National University, Gwangju, South Korea
Phytopathology 98:S81

Root colonization by *Pseudomonas chlororaphis* O6 in cucumbers elicited an induced systemic resistance (ISR) against *Corynespora cassiicola*. In order to gain insight into O6-mediated ISR, a suppressive subtractive hybridization technique was applied and resulted in the isolation of a cucumber galactinol synthase (*CsGolS1*) gene. The transcriptional level of *CsGolS1* and the resultant galactinol content showed an increase 12 hours earlier under O6 treatment than in the water control plants following *C. cassiicola* challenge, whereas no difference was detected in the plants without a pathogen challenge. The *CsGolS1*-overexpressing transgenic tobacco plants demonstrated constitutive resistance against the pathogens, *Botrytis cinerea* and *Erwinia carotovora*, and they also showed an increased accumulation in galactinol content. Pharmaceutical application of galactinol enhanced the resistance against pathogen infection and stimulated the accumulation of defense related gene transcripts such as *PR1a*, *PR1b*, and *NtACS1* in wild-type tobacco plants. Both the *CsGolS1*-overexpressing transgenic plants and the galactinol-treated wild-type tobacco plants also demonstrated an increased tolerance to drought and high salinity stresses.

Microscopy and microarray analyses of host response of sweet orange (*Citrus sinensis*) to *Candidatus* Liberibacter asiaticus infection
J. KIM (1), U. S. Sagaram (1), J. K. Burns (1), N. Wang (1)
(1) Citrus Research and Education Center, University of Florida, Lake Alfred, FL
Phytopathology 98:S81

Citrus greening or Huanglongbing (HLB) is one the most devastating diseases of citrus caused by a phloem limited fastidious prokaryotic alpha-proteobacterium *Candidatus* Liberibacter spp. In this work we have analyzed the colonization of the phloem by the HLB bacterium and the anatomical changes of phloem caused by HLB infection with light microscopy and transmission electron microscopy, and investigated the effect on phloem transportation of key photoassimilates. Microscopy analysis indicated that the HLB infection caused phloem disruption, and sieve pores plug which eventually blocked the photoassimilate transport. Further investigation of the host response using microarray indicated that the HLB infection significantly affected expression of 624 genes associated with sugar mechanism, plant defense, phytohormones, cell wall as well as 14 other gene categories.

Proteomic analysis of GacS-regulated proteins in a plant beneficial rhizobacterium, *Pseudomonas chlororaphis* O6
C. Kim (1), B. Kang (2), K. Yang (1), B. Cho (1), Y. KIM (1)
(1) Department of Plant Biotechnology and Biotechnology Research Institute, Chonnam National University, Gwangju 500-757, Korea; (2) Jeonnam Agricultural Extension Service Center, Naju 520-715, Jeonnam, Korea
Phytopathology 98:S81

Pseudomonas chlororaphis O6 a nonpathogenic root colonizing bacterium, induces systemic resistance against several plant diseases and suppresses plant pathogens via the production of secondary metabolites that inhibit the growth of plant pathogens. Our previous works showed that the GacS sensor kinase of *P. chlororaphis* O6 governs the production of secondary metabolites and the production of microbial determinant of induced systemic resistance, 2R,3R-butanediol. In order to identify the proteins regulated by the GacS sensor kinase, 2-D gel electrophoresis analysis total proteins from wild-type, the GacS mutant and the complemented GacS mutant were performed total 23 down-regulated protein spots were determined by MALDI-TOF and Q-TOF analysis. Genes encoding down-regulated protein spots were cloned by PCR and transcriptional expression of each gene was examined in wild-type, the GacS mutant, and the complement GacS mutant by RT-PCR analysis. Meanwhile, 17 of 19 cloned genes were regulated by GacS sensor kinase at transcriptional level, but outer membrane protein and LysM domain were not regulated at transcriptional level. Expression of an anthranilate para-aminobenzoate syntheses component I gene, which involves in biosynthesis of tryptophan from chorismate, was significantly decreased in the GacS mutant. In addition, Expression of tryptophan halogenase involving in biosynthesis of pyrrolnitrin, which is a secondary metabolite suppress phytopathogenic fungi, was also significantly reduced in the GacS mutant. Our genetic and biochemical analysis indicated that production of pyrrolnitrin by *P. chlororaphis* O6 is regulated by the sensor kinase GacS at the transcriptional level.

Two putative hexose kinase genes, *HXK1* and *HXK2*, are involved in FB_1 biosynthesis of *Fusarium verticillioides*
H. KIM (1), C. P. Woloshuk (1)
(1) Purdue University, West Lafayette, IN, USA
Phytopathology 98:S81

Fusarium verticillioides, which can infect maize kernels at any stage of development, must perceive and respond to the precise nutrients that are available at the time of infection. Recently, we found that greater amounts of FB_1 are produced in mature kernels than in immature kernels, suggesting that the starch component of mature kernels provides an environment that is conducive for FB_1 production. In this study, we identified two genes, *HXK1* and *HXK2*, encoding putative hexose kinases of *F. verticillioides*, and hypothesize that these genes are involved in the regulation of sugar catabolism with a role in the regulation of FB_1 biosynthesis during pathogenesis of maize kernels. We created disruption mutants of the hexose kinases (delta*hxk1* and delta*hxk2*) in *F. verticillioides*. Analysis of ergosterol content indicated that the growth of both mutants on maize kernels is similar to the wild type. However, FB_1 production of delta*hxk1* was less that 20% of the wild type and delta*hxk2* did not produce FB_1. Preliminary results also indicated that delta*hxk1* fails to grow on fructose medium and delta*hxk2* grows similar to the wild type. Furthermore, conidiation of delta*hxk2* was reduced severely when grown on either glucose or fructose medium. These results clearly indicate that growth, fungal development, and FB_1 production are affected by these kinases. We will present results from additional experiments that demonstrate how *HXK1* and *HXK2* affect the expression of genes involved in the sugar catabolism and FB_1 biosynthesis.

Ophiostomataceae associated with the exotic bark beetle, *Hylurgus ligniperda* (Coleoptera: Scolytidae), in California

S. KIM (3), T. C. Harrington (3), M. L. Flint (2), D. Liu (2), J. C. Lee (2), S. J. Seybold (1)
(1) Chemical Ecology of Forest Insects, Pacific Southwest Research Station, USDA Forest Service, Davis, CA 95616; (2) Department of Entomology, University of California, Davis, CA 95616; (3) Department of Plant Pathology, Iowa State University, Ames, IA 50011-3221
Phytopathology 98:S82

Hylurgus ligniperda (F.) (Coleoptera: Scolytidae) is native to Europe but has been introduced to Chile, South Africa, and New Zealand, and recently to Southern California in 2003. This bark beetle is a common vector of Ophiostomataceae, some of which have been considered tree pathogens or as causes of blue-stain. In this study, we identified and characterized Ophiostomataceae associated with introduced populations of *H. ligniperda* in California. Adult beetles were reared from infected pine logs at two California sites. Adult beetles were cut into four sections and plated in malt agar with streptomycin and cycloheximide, which selects for species of *Ophiostoma* and related anamorph genera. In total, 12 species of fungi belonging to the Ophiostomataceae were isolated from *H. ligniperda*. Similar to other locations, the most frequently isolated species in California were *O. ips* and *O. galeiforme*, which were isolated from 26% and 23%, respectively, of the 118 beetles. *Ophiostoma ips* was dominant at the Bonelli Park site, and *O. galeiforme* was dominant at Descanso Gardens. The other isolated species were *O. piceae* (from 9% of the beetles), *O. querci* (8%), *O. huntii* (7%), and *Sprothrix* sp. (5%); *O. floccosum*, *O. stenoceras*, *O. nigrocarpum*, *O. pluriannulatum*, *Leptographium truncatum*, and *L. serpens* were isolated from less then 5% of the beetles. Most of these species were already known in the United States and have been found in association with *H. ligniperda* in other countries.

First report of *Phytophthora hedraiandra* in Pennsylvania

S. KIM (1), E. V. Nikolaeva (2), S. Park (2), S. Kang (2)
(1) PA Dept. of Agriculture, Harrisburg, PA, USA; (2) Penn State, University Park, PA, USA
Phytopathology 98:S82

Phytophthora hedraiandra has been recently identified for the first time in PA. The pathogen was isolated from *Rhododendron catawbiense* showing twig blight symptoms collected from three different PA counties from June 15 to July 22, 2004 as part of the PPQ national *P. ramorum* survey. The pathogen was isolated from 12 samples comprised of 'Roseum Pink,' 'Roseum Elegans,' and unknown cultivars that originated from Virginia, Oregon, and unknown sources. The cultures grown on PARP at 20°C were tentatively identified as *P. cactorum* by morphology, and stored in hemp-seed water (one culture plug from V8-200/two seeds/10 ml sterile distilled water). Lately, the pathogen was identified as *P. hedraiandra* based on its sequences of the ITS region. Koch's postulate was satisfied by inoculating *R. catawbiense* 'Roseum Pink' using the 12 isolates of *P. hedraiandra*. A detached leaf on moistened paper towel in a plastic bag was inoculated with a 5-mm disc of the inoculum on V8 200 agar and then incubated at 27°C for 7 days, 12 h light cycle (Each isolate replicated 4x). The lesion size averaged 1,215 mm^2 (SD ± 606 mm^2). No symptoms were observed on control leaves that were inoculated with V8 200 agar plugs. *P. hedraiandra* was reisolated from the lesions. This first detection of *P. hedraiandra* in PA is from limited areas and further study is needed for comprehensive risk assessment.

Dissecting the responses to the *Pectobacterium* type III secretion system in two *Nicotiana* species

H. KIM (1), A. O. Charkowski (1)
(1) University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 98:S82

The role of the T3SS in soft rot *Pectobacterium* is unclear, although it has been implicated in pathogenesis and triggering non-host resistance. To investigate the role of T3SS in a highly pathogenic *P. carotovorum* strain, we constructed mutations in *hrpL*, the T3SS sigma factor, and *hrpN*, *hrpW*, and *dspE*, which encode T3SS-secreted proteins. When inoculated into *Nicotiana benthamiana* leaves, *P. carotovorum* elicited a T3SS-dependent plant cell death similar in appearance to a hypersensitive response, however disease symptoms spread from the initial lesion within 48 hrs. Among the T3SS mutants, only the *dspE* and *hrpL* mutants were affected in ability to cause plant cell death and subsequence spread of symptoms in *N. benthamiana*. The *Pseudomonas syringae dspE* homolog, *avrE*, has been reported to suppress callose formation in leaves. Intriguingly, we observed the formation of callose upon infiltration of both wild type and T3SS mutants in *Nicotiana tabacum*, thus *P. carotovorum* does not suppress callose in *N. tabacum* leaves. Although the *P. carotovorum hrpL* mutant was required for elicitation of plant cell death, we observed no difference in bacterial growth between the *hrpL* mutant and the wild type over the course of five days in *N. benthamiana* leaves when low levels of bacteria were inoculated into leaves. Similarly, an *outD* mutant, which is unable to secrete the plant cell wall degrading enzymes that cause the soft rot symptoms characteristic of *P. carotovorum*, also grew in *N. benthamiana* leaves. However, a *hrpL outD* double mutant had at least a 10-fold reduction in bacterial growth, suggesting overlapping contributions to bacterial growth.

Variation in nutrient utilization and sensitivity to antibiotic inhibition within a global collection of Streptomyces scabies

L. KINKEL (1), J. Kang (1)
(1) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S82

Potato scab, caused by Streptomyces scabies, can cause significant economic losses on potato. Antibiotic-producing, non-pathogenic Streptomyces isolates have shown promise in controlling scab, though scab biocontrol remains inconsistent in field trials. We hypothesize that variation in scab biocontrol reflects variability in the pathogen population in nutrient utilization profile and sensitivity to antibiotic inhibition. We determined nutrient utilization profiles for a collection of n = 90 pathogen isolates collected from locations around the world using Biolog SF-P2 plates. In addition, sensitivity of each pathogen isolate to antibiotic inhibition against a collection of 5 different inhibitory Streptomyces was quantified. There were significant differences among pathogen isolates in nutrient utilization profiles and in the efficiency of nutrient use. Furthermore, pathogens from different locations varied significantly in resistance to antibiotic inhibition. Relationships between nutrient utilization and sensitivity to antibiotic inhibition among isolates provide insight into the competitive strategies of different pathogen isolates. These data suggest that pathogen populations vary widely in their potential sensitivity to resource competition- or antibiotic inhibition-based biological control. A clear understanding of the local pathogen population, and especially its sensitivity to inhibition or competitive exclusion, is needed to optimize the development of effective scab biocontrol.

***Stigmina lautii* appears to have replaced *Rhizosphaera kalkhoffii* on spruce in North Dakota**

K. M. KINZER (1), J. A. Walla (1)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND 58105
Phytopathology 98:S82

Stigmina lautii Sutton was discovered on spruce needles in ND in 2006, but pathogenicity of *S. lautii* has not been proven. Our objective was to characterize *S. lautii* distribution and incidence compared with that of *Rhizosphaera kalkhoffii* Bubak. Identity of potential needle pathogens on spruce trees observed in eastern and central ND and on spruce needlecast samples received by the NDSU Plant Diagnostic Lab was determined. Sporodochia and spores of *S. lautii* were observed on samples from spruce trees exhibiting needlecast in 12 counties of eastern and central ND in 2006 and 2007. Hosts were Colorado spruce (*Picea pungens* Engelm.) and white spruce (*P. glauca* Moench). Of 20 spruce samples with needlecast received or collected in 2006 and 2007 in ND, only *S. lautii* was found as a possible cause on 19, while only *R. kalkhoffii* was identified on one. Previously, *R. kalkhoffii* had been microscopically confirmed in eastern and central ND, and needlecast had subsequently commonly been attributed to this fungus based on symptoms and presence of darkly pigmented fruiting bodies in needle stomates (with magnification up to 40X). Similarities in appearance of fruiting bodies of *R. kalkhoffii* and *S. lautii* likely resulted in some previous mistaken diagnoses when spores were not observed to confirm identity. *S. lautii* now appears to be the prevalent fungus on spruce needles in ND. The discovery of *S. lautii* on spruce in ND has led to indecision in dealing with diseased spruce compared to when only *R. kalkhoffii* was associated with spruce needlecast because, to our knowledge, justification for and means of management of *S. lautii* have not been demonstrated.

Linking models of resource-based tradeoffs in trees: An assessment of growth, defense, carbon allocation patterns, and potential ectomycorrhizal regulation in paper birch

N. M. KLECZEWSKI (1), P. Bonello (1)
(1) The Ohio State University
Phytopathology 98:S82

Resource availability affects tradeoffs between primary and secondary metabolism such that rapidly growing trees characteristic of fertile soils have lower levels of secondary metabolites and vice versa. Decreased accumulation of secondary metabolites increases susceptibility of trees to disease and drought stress. Soil fertility levels also are often negatively correlated with ectomycorrhizal (EM) abundance on tree root systems, which decreases the ability of trees to obtain water and limiting nutrients. The relationship between metabolic tradeoffs in response to soil fertility and regulation of EM is unknown. We studied metabolic tradeoffs, internal carbon allocation, and EM

associations of paper birch seedlings in a controlled greenhouse setting. A fully factorial design with soil type, fertilization, and drought as treatments was used. Overall growth, carbon allocation, soluble carbohydrates, soluble phenolics, and lignin were measured for foliage and root material. Seedling growth rates increased with fertilization, while levels of foliar phenolics and root-to-shoot ratios decreased. Root phenolics and lignin decreased upon fertilization of subsoil, but increased in fertilized topsoil. EM abundance was strongly negatively correlated with levels of root phenolics and lignin, indicating that the host may regulate its EM associations by manipulating these general defense responses in complex interactions with resource availability.

Development of a rapid pathogenicity assay for *Verticillium dahliae* using early flowering lettuce

S. J. KLOSTERMAN (1), R. J. Hayes (1)
(1) USDA-ARS, 1636 E. Alisal St., Salinas, CA 93905
Phytopathology 98:S83

Conventional assays of Verticillium wilt on lettuce require approximately three months from the time of seeding. A reduction in time required for analyses of symptom development would be useful in studies of the *Verticillium dahliae*-lettuce interaction. In this study, a growth chamber assay was evaluated for the rapid assessment of symptoms on lettuce. Two-week seedlings of La Brillante, Salinas, and the early flowering plant introduction (PI) 251246 were either non-inoculated or inoculated in tubes with *V. dahliae* conidial suspensions. The suspensions contained race 1 and race 2 isolates of *V. dahliae* at concentrations of 0, 1×10^3, 1×10^5, and 1×10^7/ml. Percent symptomatic leaves were recorded at 14 and 21 days, and root discoloration at 21 days. Symptoms were concentration dependent in Salinas and PI 251246, while symptom development in La Brillante was dose and race dependent, with race 2 causing significantly more root discoloration than race 1 at 1×10^7/ml. PI 251246 developed consistent leaf and root symptoms at 21 days following inoculation with race 1 and race 2 at 1×10^7/ml. Because cultivars Salinas and La Brillante exhibited inconsistent foliar symptoms and limited numbers of plants with root discoloration, the method is not suitable for selecting cultivars for resistance to Verticillium wilt. However, the use of the early flowering line PI 251246 in the tube assay provides a convenient and rapid method to analyze virulence of *V. dahliae* on lettuce.

Structural dynamics of NDR1 function in mediating plant resistance to Pseudomonas syringae

C. P. KNEPPER (1), E. Savory (1), B. Day (1)
(1) Michigan State University, East Lansing, MI
Phytopathology 98:S83

Arabidopsis NDR1 (non-race specific disease resistance-1) plays essential roles in resistance mediated by CC-NBS-LRR class of resistance proteins, among which include RPS2, RPM1 and RPS5. Upon infection with Pseudomonas syringae pv. tomato DC3000 expressing bacterial effector proteins AvrRpt2, AvrB/AvrRpm1 and AvrPphB, resistance is activated by the aforementioned resistance proteins. NDR1 is a plasma membrane-localized, glycosylphosphatidyl-inisotol (GPI)-anchored protein. While the expression of NDR1, as well as the genetic requirement for NDR1 in plant disease resistance has been detailed, the specific biochemical mechanism(s) of NDR1 function is largely unknown. Using homology modeling and structure threading to predict the tertiary structure of NDR1, the structure of Arabidopsis LEA14 was identified as having a significant structural probability match to NDR1. This has led to the use of site-directed mutagenesis targeting the omega-site of NDR1, as well as conserved motifs known to be required for LEA14-like proteins, to elucidate a role for NDR1 in resistance signaling. Analysis of several site-directed mutants has yielded new information as to the effects of orientation and dimerization in NDR1 function. This includes the use of vectors expressing fragments of NDR1 to identify the residues critical for dimerization. The use of EMS mutagenesis, as well as activation tagging mutagenesis screens, have allowed us to further investigate the role of NDR1 in disease resistance signaling. Collectively, the experiments presented here will allow us to identify complementary mutations leading to the characterization of NDR1 in the R-gene mediated defense response pathway.

Effect of the infection times by *Zucchini yellow mosaic virus* on the yield and growth in cucumber

S. KO (2), M. Kim (2), M. Cho (2), Y. Lee (1), H. Choi (1), G. Lim (2)
(1) Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon, Korea; (2) Vegetable Experiment Station, Jeonnam Agricultural Research and Extension Services, Kurye, Jeonnam, Korea
Phytopathology 98:S83

We investigated the effect of the infection times of *Zucchini yellow mosaic virus* (ZYMV) on the growth and yield of cucumber plants at the semi-forcing culture and the retarding culture in 2007. When cucumber was inoculated with ZYMV at transplanting time, 15, 30, 45, 60, and 75 days after transplanting, vine length, internode length, number of leaf of the plants and marketable yield largely decreased as the cucumber infected earlier. The regression models were obtained between the disease incidence levels at 20 days after transplanting and the marketable yield of cucmumber: $y = 9333.1^{e^{-0.0317x}}$ ($R^2 = 0.8946$) at the semi-forcing culture and $y = 14695^{e^{-0.0303x}}$ ($R^2 = 0.8735$) at the retarding culture. And the cucumber yield loss regression models between the ZYMV infection days from the final harvesting time and the rates of yield decrease were expressed as $y = 1.0851x-6.7067$ ($R^2 = 0.9567$) at the semi-forcing culture and $y = 1.0439x+2.1321$ ($R^2 = 0.9674$) at the retarding culture.

Analysis of genes involved in biofilm formation of *Erwinia amylovora*: Implications in pathogenesis

J. M. KOCZAN (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI 48824
Phytopathology 98:S83

Erwinia amylovora is the causal agent of fire blight, a disease that is particularly damaging on rosaceous species, such as apple and pear. Current methods of control focus on chemical and antibiotic treatments. The popularity of highly susceptible cultivars has driven the need to find other methods to control fire blight. One approach is to study the molecular mechanisms by which the pathogen causes disease. Previous research has shown that *E. amylovora* forms a biofilm; a biofilm is surface-associated complex aggregation of bacteria, exopolysaccharides and macromolecules, which may confer protection against harsh environments and contribute to pathogenicity. Amylovoran, one of the exopolysaccharides (EPS) produced by *E. amylovora*, has been shown to be required for both disease and biofilm formation. We screened a transposon mutagenesis library of *E. amylovora* Ea1189 and used a bioinformatics approach to identify candidate genes that are involved in biofilm formation and surface attachment. Potential mutants have been initially screened for biofilm formation using a well established crystal violet staining method. Further characterization includes examination using microscopy, specifically scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) using an *in vitro* flow cell. The mutants have also been screened for their ability to cause disease using an immature pear fruit assay and shoot assay on apple trees. Results of these findings will be presented as well as implications for the roles these genes may play in pathogenicity.

Controlling dollar spot: Climatic conditions and the timing of fungicide applications

J. L. KOENIG (1), L. V. Madden (1), T. E. Hicks (1), M. J. Boehm (1)
(1) The Ohio State University Plant Pathology Department, Columbus, OH, USA
Phytopathology 98:S83

Effective control of dollar spot (caused by *Sclerotinia homoeocarpa*) typically requires repeated and timely applications of fungicides. Previous research has shown that the application of fungicides to asymptomatic turfgrass in the fall and/or spring may reduce dollar spot severity the following season. The impact of these preventive fungicide applications varied by site and over time. In 2006, replicated field studies were established at two locations to determine the relationship between climatic conditions and the ideal time for making preventive fungicide applications. Weather monitoring stations were installed at both locations to record on-site soil moisture, soil temperature, air temperature, precipitation and relative humidity. Sequential applications of propiconazole and chlorothalonil as a combination treatment were applied to asymptomatic fairway turfgrass in the fall of 2006 and the spring of 2007. Disease severity, assessed as the number of dollar spot infection centers per plot, was recorded through July 2007 and used to calculate an AUDPC for each treatment. In general, fall fungicide applications had no impact on disease severity. Applications made in mid-to-late April significantly reduced disease severity. The average monthly temperature between October 2006 and January 2007 was 2.8C warmer than the 30-year average for the same period. The lack of disease suppression observed with the fall 2006 fungicide applications in this study is consistent with previous reports indicating that climatic conditions significantly impact fungicide efficacy.

Population dynamics and dispersal gradient of *Aphelenchoides fragariae* in ornamental nurseries

L. M. KOHL (1), C. Y. Warfield (2), M. Benson (1)
(1) North Carolina State University; (2) University of California-ANR
Phytopathology 98:S83

Foliar nematodes (*Aphelenchoides fragariae*) cause aesthetic damage and defoliation of nursery-grown woody ornamental crops. The influence of environmental factors on the population dynamics of foliar nematodes was measured at a container nursery in North Carolina in 2006 and 2007. Air temperature, relative humidity, and rainfall data were recorded each season. Symptomatic leaves were removed every 2 weeks, from May until October,

from a block of 30 lantana plants (*Lantana camara*) naturally-infected with foliar nematodes. Leaf samples were cut into pieces and incubated in a minimal amount of deionized water at room temperature. After 2 days foliar nematodes that had emerged were counted. The population of nematodes per gram of sampled leaf tissue increased as the season progressed, reaching a peak in July of each year, before declining for the remainder of the summer. However, a second peak in the nematode population occurred in October 2007. Changes in the nematode population were positively correlated with rainfall and daily high temperatures, but had no significant correlation with average or low daily temperatures, or with relative humidity. The dispersal gradient of foliar nematodes was evaluated in an experimental nursery block by spacing healthy plants at a distance of 0 (touching), 30, or 100 cm from an *A. fragariae*-infected plant. Distances were maintained by re-spacing plants as needed. Plants were overhead irrigated twice daily, and five rainfall events occurred during the trial. After 11 weeks 100%, 10% and 5% of the healthy plants were infected at the 0-, 30- and 100-cm distances, respectively. Increased plant spacing between nursery blocks may effectively reduce the spread of foliar nematodes within a growing facility.

Can rotational crops, weeds, and native plants support *Fusarium virguliforme* populations in the absence of soybeans?

T. M. KOLANDER (1), J. E. Kurle (1), D. K. Malvick (1)
(1) University of Minnesota, Department of Plant Pathology, St. Paul, MN, USA
Phytopathology 98:S84

Sudden death syndrome (SDS), caused by *Fusarium virguliforme* (*Fv*) (formerly *F. solani* f. sp. *glycines*) is a yield-limiting disease of soybean in the U.S. Inoculum of *Fv* is soilborne and persistent following various crop rotation regimes. Previous greenhouse studies indicated a host range for *Fv* that is limited to soybean (*Glycine max*), mung bean (*Vigna radiata*), and green bean (*Phaseolus vulgaris*) when plants were not wounded, however, lima bean (*P. lunatus*), and cowpea (*V. unguiculata*) were hosts when plants were wounded. Our objective was to determine the symptomatic and asymptomatic host range of *Fv* for crops, weeds, and native plant species, with the initial focus on crops that may be grown in rotation with soybean. Corn (*Zea mays*), wheat (*Triticum aestivum*), sugar beet (*Beta vulgaris*), perennial ryegrass (*Lolium perenne* L.), pea (*Pisum sativum*), and navy and pinto bean (*P. vulgaris*) were inoculated in a greenhouse with a layer of sorghum infested with *Fv* that was placed 1 cm below the seeds. After 5 to 6 weeks of growth, root and shoot symptoms were assessed, total plant fresh biomass was measured, and root sections cultured to reisolate the pathogen. DNA was extracted from the remaining root tissue and quantitative PCR was performed using *Fv*-specific primers. Based on root rot symptoms and/or quantities of *Fv* DNA in the roots, pinto bean, navy bean, wheat, and corn may be susceptible to infection or support growth of *Fv*. Preliminary results suggest an expanded host range for *Fv* that includes potential asymptomatic plant hosts capable of sustaining or supporting pathogen survival and reproduction in the absence of soybeans.

Evaluation of cover crops for management of Phytophthora blight on squash

D. KONÉ (1), A. S. Csinos (1), J. Yin (1), P. Ji (1)
(1) University of Georgia, Tifton, GA, USA
Phytopathology 98:S84

The efficacy of *Brassica* cover crops that produce generic biocides for management of Phytophthora blight on squash caused by *Phytophthora capsici* was evaluated. Fresh roots and leaves of selected *Brassica* species were used separately as amendments of field soil artificially infested with *P. capsici* in greenhouse studies. Squash seedlings were transplanted in the soil amended with the cover crops at different soil/cover crop ratios. Field soil without amendment of cover crops was used as a control. Disease incidence was recorded weekly until three weeks after transplanting and quantified as the percentage of squash plants showing Phytophthora blight symptoms. The leaves of canola and radish were more effective than the roots in reducing disease incidence, with more than 65% disease reduction when the soil was amended with the leaves at 0.5% (w/w). Both the leaves and roots of mustard, when incorporated into the soil at 1% and 5% (w/w), reduced disease incidence significantly compared with the non-treated control. In laboratory studies, roots and leaves of *Brassica* cover crops placed in closed plastic containers reduced growth of *P. capsici* on agar plates that were placed in the same containers. The results indicated that some *Brassica* cover crops had the potential to suppress *P. capsici* and disease development on squash when used as soil amendment. Field studies are in progress to evaluate the effect of soil amendment with selected *Brassica* cover crops on populations of *P. capsici* in the soil and subsequent disease development on squash.

Assessment of foliar and root diseases of banana and plantain in Georgia

D. KONÉ (1), P. Ji (1), E. G. Fonsah (1), A. S. Csinos (1)
(1) University of Georgia, Tifton, GA, USA
Phytopathology 98:S84

Bananas are susceptible to a variety of plant pathogens, however, diseases that occur on bananas in Georgia have not been characterized. Experiments were conducted to assess foliar and root diseases of bananas at the University of Georgia (UGA) Bamboo Farm and Coastal Gardens in Savannah, GA. Samples of roots and leaves were collected from 34 cultivars of banana belonging to diploids, triploids and tetraploids groups. Roots were washed to remove soil for description of symptoms. Small lesions and other symptoms were present on the roots of all the cultivars. Epidermis, cortex and rootlets were used separately for fungi isolation and selective media were used for isolation of species of *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora*. Fungi associated with symptomatic roots were *Fusarium* (89.20%), *Rhizoctonia* (36.79%), and *Pythium* and *Phytophthora* (4.91%). Species of *Fusarium* were more frequently isolated from cortex while *Rhizoctonia* species were more frequently isolated from rootlets. An unknown fungus was isolated from epidermis (10.55%), cortex (6.49%), and rootlets (9.44%). Its identification is in progress. Spot, streak, and other symptoms were observed on youngest leaves. Fungi belonging to *Mycosphaerella* sp., *Cladosporium* sp. and *Deightoniella torulosa* were isolated from symptomatic leaves. Studies to establish the relationship between these fungi and the foliar and root diseases are being conducted under greenhouse conditions by fulfilling Koch's postulates.

Effect of non-aerated compost teas on foliar fungal pathogens of tomato

S. B. Koné (1), A. Dionne (1), R. J. TWEDDELL (1), H. Antoun (1), T. J. Avis (1)
(1) Centre de recherche en horticulture, Université Laval, Québec, QC, Canada
Phytopathology 98:S84

Compost teas are aqueous extracts of compost that have revealed suppressive activities toward plant pathogens. However, few studies have specifically tested the effect of the composition of the compost used to fabricate the tea on the reduction of plant pathogen development. In this study, the suppressive effect of five non-aerated compost teas produced from different composts was evaluated on four foliar fungal pathogens of tomato (*Lycopersicon esculentum*). *In vitro* experiments on mycelial growth of three pathogens were realized with (i) native, (ii) autoclaved and (iii) microfiltered compost teas. Autoclaved or microfiltered compost teas did not inhibit mycelial growth of the tested pathogens while native teas from chicken, sheep, and cow manure composts, and shrimp compost showed a significant suppressive effect on the mycelial growth of *Botrytis cinerea*, *Alternaria solani*, and *Phytophthora infestans* when compared to the water control. A greenhouse experiment carried out with weekly applications of native compost teas showed that teas fabricated from chicken and sheep manure composts demonstrated a marked reduction of powdery mildew (*Oidium neolycopersici*) severity on the tomato leaves. Results showed that the efficacy of compost tea was a function of the type of compost used in its fabrication.

Zoospore responses to environmental pH of seven *Phytophthora* species commonly isolated from irrigation reservoirs at ornamental plant nurseries

P. KONG (3), G. Moorman (1), J. Lea-Cox (2), D. Ross (2), S. Umesha (3), P. Richardson (3), C. Hong (3)
(1) The Penn State; (2) University of Maryland; (3) Virginia Tech
Phytopathology 98:S84

Phytophthora species are commonly referred to as water molds. However, there is little information about how long and at what rate individual species may survive aquatic environments that often undergo significant daily and seasonal changes chemically, physically and biologically. In the present study, the responses of zoospores to a range of pH from 3 to 11 were tested in seven species that are frequently isolated from nursery irrigation reservoirs including *P. citricola*, *P. citrophthora*, *P. drechsleri* (Dre II), *P. irrigata*, *P. megasperma*, *P. nicotianae* and P. *tropicalis*. Hoagland's solution at 15% strength was the primary test medium. Additional tests using irrigation water as the medium were performed for *P. citrophthora*. Aliquots of a zoospore suspension were added to the test media pre-adjusted to pH levels of 3, 5, 7, 9 and 11 with NaOH or HCl. Infested media were incubated in the dark at room temperature for 0, 1, 3, 5, 7 days. Then, 1 ml was plated in 10-cm Petri dishes with PARP-V8 agar. Emerging colonies were counted after 48–72 h culture and pH responses were assessed by number of days on which zoospores remained recoverable and rate of survival (calculated by dividing colony-forming units in each replicate dish by that in optimal treatment of the same species). The longest survival was 7 days at pH 3 and 11 for *P. citricola* and *P. drechsleri* and at pH 3 only for the remaining species tested. The greatest recovery rates were observed always in dishes of day 0 at a range of pH 5 to 9, depending on species. Comparatively, *P. citrophthora* survived longer and at a higher rate in irrigation water than in Hoagland's solution. The underlying mechanisms and implications of these zoospore responses are discussed.

Quorum sensing operates in *Phytophthora nicotianae*
P. KONG (1), C. Hong (1)
(1) Virginia Tech
Phytopathology 98:S85

The term quorum sensing was introduced to describe the control of gene expression in bacteria species in response to cell density. Bacteria produce, detect and respond to hormone-like signal molecules called autoinducers to coordinate communal behaviors. Most autoinducers (e.g. acyl-homoserine lactones, AHL) promote intraspecies communication, but autoinducer 2 (AI-2) allows interspecies communication and regulates gene expression of many important behaviors including virulence. Apart from bacteria, no organism has been shown to have a quorum-sensing system involving AI-2. Here we show operation of quorum sensing involving AI-2 in *Phytophthora nicotianae*. Using two autoinducer reporters, we demonstrated that zoospores produce an AI-2-like signal but not AHL. We also demonstrate chemical communication among zoospores prior to or during plant infection by *P. nicotianae*. Autoaggregation and plant infections that usually require a high concentration of zoospores occurred at a low concentration or single spore level when provided with zoospore free fluid (ZFF) from a highly concentrated suspension. Moreover, zoospores at low concentration did not move toward to plant tissue unless supplied with ZFF. These results indicated *Phytophthora* species may share a similar quorum sensing mechanism with bacteria although their autoinducers may be produced through different pathways. This mechanism may allow *Phytophthora* species to maximize infection potential by use of widespread bacterial autoinducer (AI-2) in nature. It may thus be possible to develop novel methods to control Phytophthora diseases through interfering with the pathogen's communication systems.

Management of whitefly-transmitted viral watermelon vine decline in Florida
C. S. KOUSIK (1), S. T. Adkins (2), W. W. Turechek (2), P. D. Roberts (3)
(1) U.S. Vegetable Laboratory, USDA-ARS, Charleston, SC, USA; (2) USHRL, USDA-ARS, Ft. Pierce, FL, USA; (3) University of Florida/IFAS, SWFREC, Immokalee, FL
Phytopathology 98:S85

Watermelon vine decline (WVD) caused by the whitefly transmitted Squash vein yellowing virus (SqVYV, family: Potyviridae) has been a major limiting factor in watermelon (*Citrullus lanatus*) production in southwest and west-central Florida for the past several years. Symptoms of WVD typically manifest as sudden decline of vines at harvest time or one to two weeks prior to harvest and can also affect fruit quality. The combination of reflective plastic mulch and chemical treatments for management of whitefly on WVD development was evaluated during fall of 2006 and 2007. The chemical treatment consisted of an Admire (Imidacloprid) drench at transplanting followed by two sprays of Oberon (Spiromesifen). Virus inoculum was introduced by planting SqVYV-infected squash plants at the ends of each plot. No significant interactions between plastic mulches and chemical treatments to manage whitefly were observed on WVD development in either year. In 2006, the chemically treated plots had significantly ($P = 0.038$) less fruits with WVD symptoms compared to the untreated plots. In 2007, the areas under disease progress curve for WVD was significantly lower for the plots chemically treated ($P = 0.0038$) for managing whitefly and the reflective plastic mulch plots ($P = 0.0214$) compared to the untreated and non-reflective mulch plots, respectively. The chemically treated plots had significantly ($P = 0.0124$) less fruits with WVD symptoms compared to the untreated plots. Our results suggest that management of whitefly can help in managing WVD. In addition, removal of recently identified SqVYV reservoir hosts such as balsam-apple and creeping cucumber near watermelon fields may further aid in the overall management of WVD in Florida.

Application of loop-mediated isothermal amplification method (LAMP) for detection of the bacterial wilt pathogen *Ralstonia solanacearum* in environmental samples
R. KUBOTA (1), M. L. Paret (1), A. M. Alvarez (1), D. M. Jenkins (1)
(1) University of Hawaii at Manoa, Honolulu, HI, USA
Phytopathology 98:S85

DNA-based detection methods can provide improved specificity and sensitivity compared to serological methods. However, these methods remain impractical for most field applications due to difficulties in concentrating the organism from the environmental samples and ensuring that enough DNA is available for detection. Isothermal DNA amplification techniques, such as Loop-mediated isothermal AMPlification (LAMP), might be suitable for rapid field detection because of its ability to amplify DNA with high specificity, efficiency, and speed without thermal cyclers. The objective of this research was to develop a rapid detection method for *Ralstonia solanacearum* (Rs) in environmental samples. To demonstrate the application of LAMP in environmental samples, edible ginger plants (*Zingiber officinale*) were infected with Rs, and effluent water samples were collected from daily irrigation water. A simple filtration technique was applied to concentrate bacteria, followed by a species-specific LAMP and pathogen presence was visually determined as the precipitation of an insoluble pyrophosphate by-product of amplification, which increased the turbidity of the solution. The detection limit of the direct LAMP assay was between 10^3 – 10^5 CFU/ml, which is the same as PCR and more sensitive than ELISA. LAMP shows promise for not only rapid detection but also for discrimination of closely related sub population of Rs in soil and water.

Progress on the development of broad spectrum disease resistance in citrus through transformation with *CNGCcit* and *bcl-2* genes
M. KUNTA (1), M. Skaria (1), J. V. Da Graca (1), T. Mirkov (2), E. S. Louzada (1)
(1) Texas A&M University-Kingsville Citrus Center, Weslaco, TX, USA; (2) Texas AgriLIFE Research, Weslaco, TX, USA
Phytopathology 98:S85

Transgenic tomato and tobacco plants expressing human *bcl-2* transgene are known to be resistant to fungal and viral pathogens. In *Arabidopsis* and tobacco, cyclic nucleotide-gated channels (CNGCs) were reported to be involved in ion homeostasis, uptake, and transport. Moreover, in *Arabidopsis*, CNGCs were shown to play an important role in defense against pathogens. A PCR amplification product comprising *CNGCcit* open reading frame (ORF) with *Xba* I and *Eco* RI ends was generated, inserted into pRTL22 plasmid, transformed into *E. coli*, and sequenced. The 3.2 kb *Hin*d III fragment of pRTL22/*CNGCcit* containing the CaMV 35S promoter with dual enhancer, *CNGCcit*, and CaMV 35S terminator was inserted into the *Hin*d III site of pBin 34SGUS to generate pBin CNGCcit construct, and transformed into *E. coli*. The cDNA clone of bcl-2 in the pPTN binary vector was digested with *Hin*d III to release a fragment consisting of CaMV 35S promoter, *bcl-2*, and CaMV 35S terminator and inserted into the *Hin*d III site of pBin 34SGUS to generate pBin bcl-2 construct. These constructs were used in *Agrobacterium tumefaciens*-mediated transformation of sour orange rootstock and 'Rio Red', 'Ruby Red' and 'Duncan' grapefruit cultivars. The presence and expression of *CNGCcit* in the putatively transformed epicotyl segments and regenerated shoots was verified by b-glucuronidase (GUS) histochemical assays. After regeneration and shoot elongation, the putatively transformed shoots were shoot-tip grafted onto C22 (a trifoliate hybrid) or sour orange rootstock. These transgenic plants will be challenged with fungal and viral pathogens to evaluate for disease resistance.

Bacteriophage-mediated detection of *Ralstonia solanacearum*
K. KUTIN (1), D. Borthakur (1), A. M. Alvarez (1), D. M. Jenkins (1)
(1) University of Hawaii
Phytopathology 98:S85

This project aimed to exploit bacteria-bacteriophage interactions for the development of sensitive techniques for the detection of *Ralstonia solanacearum*. We coupled the rapid self-replicating ability of bacteriophages with quantitative PCR (q-PCR) in an indirect assay for *R. solanacearum*. We are also exploring the possibility of using phage lytic enzymes for the selective disruption of bacterial cell membrane for further analysis of cell contents. *Six R. solanacearum* selective bacteriophages (ISO_2, M_DS1, M_DL, S3_S, S6_S1 and S5_5) were isolated from farms on the Hawaiian island of Oahu. These were tested for their generation times and their fecundities. Based these criteria, phage M_DS1 was selected as the optimal candidate for the indirect detection of *R. solanacearum*. A combination of phage amplification and q-PCR resulted in a detection limit of 2.3 CFU/ml *R. solanacearum* after incubation for an hour with 1.9 × 106 PFU/ml M_DS1 particles. The sensitivity of the approach is being tested further with drainage water collected from pots growing ginger infected with *R. solanacearum*. The lysis gene of phage M_DS1 has been isolated and expressed as a 18 KD protein and efforts are under way to evaluate its lytic activity against *R. solanacearum* and its effect on standard methods of gene replication and detection.

Diversity, virulence and 2,4-diacetylphloroglucinol sensitivity of *Gaeumannomyces graminis* var. *tritici* isolates from Washington State
Y. KWAK (1), P. A. Bakker (3), D. C. Glandorf (2), T. Paulitz (4), D. M. Weller (4)
(1) Department of Plant Pathology, Washington State University, Pullman; (2) National Institute for Public Health and the Environment, Bilthoven, The Netherlands; (3) Plant-Microbe Interactions, Utrecht University, Utrecht, The Netherlands; (4) USDA, ARS Root Disease and Biological Control Research Unit, Pullman
Phytopathology 98:S85

2,4-diacetylphloroglucinol (DAPG)-producing *Pseudomonas fluorescens* buildup in soils that have undergone wheat or barley monoculture for >4 years resulting in take-all decline (TAD). We hypothesized that *Gaeumannomyces graminis* var. *tritici* (*Ggt*) isolates (cause of take-all) in monoculture soil can

become insensitive to DAPG. Over 184 isolates of *Ggt* were baited from roots of wheat and native grasses growing in fields with different cropping histories near Lind, Ritzville, Pullman and Almota, WA. Isolates were characterized using morphological traits, growth on a semi-selective medium, *G. graminis* variety-specific PCR primers and pathogenicity tests. The sensitivity of 152 *Ggt* isolates to DAPG was determined by colony growth on agar medium. The sensitivity to DAPG was normally distributed and ED_{90} ranged from 3.14 μg ml^{-1} for sensitive isolates to 11.1 ml^{-1} for less sensitive isolates. Three *Ggt* isolates were extremely sensitive to DAPG (ED_{90} <1 μg ml^{-1}). Isolate sensitivity to DAPG was not correlated with geographic origin or cropping history of the field. There was no correlation between virulence on wheat and geographical origin, and no correlation between virulence and sensitivity to DAPG. Unexpectedly, *G. graminis* var. *avenae* (*Gga*) comprised 25% and 95% of putative take-all pathogens from fields near Almota and Pullman that were direct-seeded to continuous wheat or barley. All *Gga* isolates were extremely tolerant (mean ED_{90} 22.44 μg ml^{-1}) to DAPG.

Promoter analysis of the cryparin gene from *Cryphonectria parasitica*
B. KWON (4), M. Kim (3), S. Park (4), J. Kim (4), H. Chung (4), K. Jahng (4), M. Yang (4), A. Churchill (1), N. Van Alfen (2), D. Kim (4)
(1) Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA; (2) Department of Plant Pathology, University of California, Davis, CA 95616, USA; (3) Division of Forest Insect and Diseases, Korea Forest Research Institute, Seoul 130-712, Korea; (4) Institute for Molecular Biology and Genetics, Research Center of Bioactive Materials, Chonbuk National University, Jeonju, Chonbuk
Phytopathology 98:S86

Cryparin is an abundant cell wall-associated hydrophobin of the chestnut blight fungus, *Cryphonectria parasitica*. Although cryparin is encoded as a single copy gene (*Crp*), it is the most abundant protein produced by this fungus when grown in liquid culture. Its accumulation is decreased remarkably, however, in *C. parastica* strains containing the double-stranded (ds) RNA virus Cryphonectria hypovirus 1. To characterize the transcriptional regulatory element(s) for strong expression and viral regulation, the transcription activity of a series of truncated cryparin promoters was measured using enhanced green fluorescent protein (EGFP) as a reporter gene. Serial deletion of the *Crp* promoter region resulted in a step-wise decrease in promoter activity, indicating a localized distribution of genetic elements in the cryparin promoter. Promoter analysis indicated two positive *cis*-acting elements, one at nt 1,282 to 907 and the other at nt 640 to 427, and a repressor region between nt 427 and 181. Among them, the promoter region between nt 1,282 and 907 appeared to be necessary for hypoviral-mediated down-regulation. An electrophoretic mobility shift assay (EMSA) on the corresponding promoter region (1,282/ 907) indicated the presence of cellular factors that interacts with 376-bp region. In addition, the binding sites were further confined within the regions located at nt 1,257 to 1,158 and nt 1,107 to 1,008, with the characteristic AGGAGGA-N42-GAGAGGA and its inverted repeat TCCTCTC-N54-TCCTCCT, respectively. These results suggest that the regulatory region between nt 1,282 to 907 contains cis-acting elements involved in high expression as well as viral regulation of the cryparin gene.

Analysis of gene expression of *Rhizoctonia solani*, (AG-4) to understand its virulence and biology
D. K. LAKSHMAN (2), N. W. Alkharouf (1)
(1) Department of Computer and Information Sciences, Towson University, Towson, MD, USA; (2) USDA-ARS, Beltsville, MD, USA
Phytopathology 98:S86

The basidiomycete *Rhizoctonia solani* (Tele: *Thanatephorus cucumeris*) is comprised of important plant pathogens, saprophytes and mycorrhizae and exists in several hyphal anastomosis groups (AGs) with distinct host specializations. AG-4 isolates cause diseases in a broad range of hosts, including ornamentals and turfgrasses. Knowledge of the molecular mechanism of Rhizoctonia pathogenicity and virulence is only rudimentary at present. It is important to develop a greater understanding of the interactions involved in pathogenicity, particularly to differentiate the genes involved in establishment of infection from those involved in maintaining an infection, as different approaches would be taken to preventing infection compared to controlling infection. A thorough knowledge in this area is also necessary to assist breeders in identifying genes that can be targeted in order to generate resistant varieties and to determine whether such resistance is likely to be strain-specific or broadly effective. In an effort to extend our knowledge of the molecular mechanism of virulence of this pathogen, we have develop two normalized EST libraries specific to a virulent isolate and a 3-O-methylglucose induced virulence-repressed isolate of *R. solani*, AG-4, isolate Rs23. Thus far, about 2000 EST clones has been sequenced and an overall analysis of the cDNA sequences will be carried out. Subsequently, more sequencing will be conducted and analyzed for differential gene expression to target pathogenicity associated genes of *R. solani*. Knowledge gained from gene expression of the AG-4 isolate will be utilized for conducting comparative genomic and proteomic studies of other AGs that infect ornamentals.

Isolation and evaluation of a new race of anthracnose on dry bean cultivars in North Dakota
R. S. LAMPPA (1), R. S. Goswami (1)
(1) North Dakota State University, Dept. of Plant Pathology, Fargo, ND 58105, USA
Phytopathology 98:S86

A Colletotrichum lindemuthianum isolate obtained from navy beans in North Dakota in 2005 was evaluated for race identification. Pathogenicity tests were conducted in the greenhouse using spore suspensions containing 106 spores/ml which were sprayed on the primary leaves of two week old seedlings. Inoculated plants were incubated in a humidity chamber for five days with 12 hours fluorescent light prior to transfer to the greenhouse. Disease rating was conducted ten days after inoculation. The virulence pattern of this isolate, on a set of standard differentials and 12 commercial cultivars, was different from races 73, 7 and 89 commonly found in the United States. The new isolate produced lesions on the differentials Michelite, PI 207262 and AB 136, a reaction typical of the C. lindemuthianum race 1153, not reported previously in the U.S. This race had been originally identified in Costa Rica and Ecuador. Interestingly, the navy beans, 'Avalanche' and 'Vista' were resistant to the new isolate while being susceptible to race 73. To our knowledge this is the first report of race 1153 in the U.S. and navy beans appear to be more resistant to this race as compared to the pinto beans commonly grown in this region.

Efficacy of control methods on black rot caused by *Xanthomonas campestris* pv. *campestris* in greenhouse transplant production
H. W. LANGE (1), C. D. Smart (1)
(1) Cornell University
Phytopathology 98:S86

Black rot caused by the bacterium, *Xanthomonas campestris* pv. *campestris* (Xcc) is often a serious disease in New York State crucifer fields. This pathogen can be seed borne and even rigorous seed testing can not guarantee every seed is free of bacteria. In greenhouse transplant operations conditions are ideal for the spread of Xcc with dense plant populations and overhead watering. Asymptomatic transplants can initiate field infections which are very difficult to control. We are investigating the efficacy of chemical and biological products to control spread of black rot in cabbage seedling trays with a centrally placed infected plant serving as an inoculum source. At 0, 7 and 14 days after placing the inoculated plant in the flat, leaves were collected from approximately 10 and 20 cm from the inoculation point. Leaf washes were used for dilution plate counts and real-time PCR to quantify pathogen numbers on asymptomatic seedlings. This comparison will allow us to determine if treatments in the greenhouse are effective at suppressing Xcc in an environment favoring disease spread, and if this will arrest black rot development when seedlings are transplanted to the field.

Compost and biological amendment effects on soilborne disease and soil microbial communities
R. P. LARKIN (1), S. Tavantzis (2), E. Bernard (2), A. Alyokhin (2), S. Erich (2), S. Gross (2)
(1) USDA-ARS, Orono, ME; (2) University of Maine, Orono, ME
Phytopathology 98:S86

Effects of compost and biological amendments on soilborne diseases and microorganisms were assessed in field trials in northern Maine under both conventional and organic potato production practices. Three different biocontrol amendments, hypovirulent *Rhizoctonia solani* Rhs 1A1 (HvRs), *Bacillus subtilis* (Bsub), and *Trichoderma virens* (Tvir), as well as a nontreated control were used in conjunction with plots both amended and not amended with a conifer-based (Hemlock bark) compost (19 Mg/ha). At the conventional site, compost amendment reduced incidence and severity of black scurf by 12–27%, and increased tuber yields by 13–23%. Biocontrol treatments (Tvir and HvRs) reduced incidence and severity of black scurf by 9–31% but had no significant effect on yield. The combined effect of compost and biocontrol amendments reduced black scurf by 30–48% and increased yield. At the organic site, where soil was already rich in organic matter, compost did not significantly reduce scurf or scab, but increased tuber yield (9–30%). Biocontrol treatments (Tvir and Bsub) reduced incidence and severity of black scurf by 10–48%, scab by 5–20%, and the total of all diseases by 15–30%. All treatments significantly affected soil microbial communities, with compost amendments generally resulting in more pronounced changes in community characteristics than biological amendments. Overall disease levels were lower and yields higher at the organic site.

Appropriate compost and biological amendments had significant positive effects on soil quality, disease reduction, and yield, and should play an important role in sustainable soil and disease management programs.

Virulence and molecular characterization of Cuban isolates from *Peronospora hyoscyami* sp. *tabacina*

S. LARRAÑAGA (3), B. Muiño (2), O. Chacon (3), J. A. Crespo (3), O. Borras (1)
(1) Laboratory of Plant Functional Genomics, Center for Genetic Engineering and Biotechnology; (2) Plant Health Institute; (3) Tobacco Research Institute, Plant Pathology Department, San Antonio de lo Baños, Habana, Cuba
Phytopathology 98:S87

Tobacco Blue Mold caused by *Peronospora hyosciami* sp. *tabacina* is the major fungal diseases that affect *Nicotiana tabacum* crops in Cuba. The aim of this work was to determine phenotypic and genotypic differences between six isolates from distinct geographic origins. We used leaf discs bioassay to analyze some phenotypic characteristic. The disks were made with leaves of 9 tobacco genotypes from Trap Collection of CORESTA. There were not significant differences between the isolates when we evaluated the phenotypic characteristic. In all cases we found that fungal virulence was modulated by cultivar resistance, the isolates were more aggressive against susceptible genotypes Bergerac and Samsoum however; they showed a reduced virulence against more resistant genotypes RXT, Chemical Mut, GA55 and Bel G110. In order to find a genetic variability among isolates we amplified and sequenced the Internal Transcribed Spacer (ITS) regions. Sequence comparisons revealed a high homology (99%) within this region. Thus, we amplified a polymorphic unknown region called 1602 by Wiglesworth in 1994 and the sequence showed significant differences between isolates according with the distinct area of collect. The isolates can be divided in 3 homology groups. Group 1 formed by the 1, 2 and 3 isolates which share 99% homology. The Group 2 include isolate 5 and 6 which are identical in 98%, they are similar from the Group 1 in 31%. Group 3 composed only by the 4 isolate that share homology sequence from group 1 and 2 in a 37 and 25% respectively the most different isolates of the six. When we compared sequences between isolates with the 1602 region we found that Group 2 showed the highest homology (96.5) whereas Group1 and 3 were 28.5% and 24% homologous with that region respectively. Genes that are expressed during tobacco-blue mold interaction (Pt 15, Pt 44 and Pt 43) were amplified and the PCR bands were showed in agarose staining gel.

A major QTL is associated with resistance to curly top virus in common bean (*Phaseolus vulgaris*) landrace G122

R. LARSEN (2), P. Miklas (2), C. Kurowski (1)
(1) Harris Moran Seed Company, Davis, CA; (2) USDA-ARS
Phytopathology 98:S87

Curly top virus (CTV) is a devastating disease of common bean in the Western U.S. Genetic resistance provides effective control but can be difficult to discern in early generations. Two populations of F5:7 RILs were derived from separate F1 seeds from a cross between the G122 landrace from India known as 'Jatu Rong' (CTV-resistant) and Taylor Horticultural (CTV-susceptible). The A and B populations comprising 98 individuals in total were evaluated for reaction to CTV in two field experiments over two years, and were screened for potential markers linked to resistance using a population of RAPD primers. Five dominant RAPD markers derived from G122 and completely linked with each other were associated with a major effect QTL that exhibited stable expression across years and populations. The phenotypic variation explained by the QTL in Population A (43.8%) was greater than in Population B (21.9%). Three additional linked RAPD markers detected a QTL with minor effect but in Population A only. Two of the three markers were polymorphic in the common bean BAT 93/Jalo EEP558 core mapping population enabling integration of the major QTL to linkage group B6. G122 resistance is independent of the *Bct* gene conferring resistance to CTV on linkage group B7, as also alluded by allelism tests. These findings indicate that G122 possesses novel resistance to CTV.

ELISA and PCR survey for *Cercospora beticola* in field soils from three Upper Midwest States of the United States

R. T. LARTEY (1), T. Caesar-TonThat (1), S. Hanson (1), R. G. Evans (1)
(1) USDA/ARS
Phytopathology 98:S87

Several fields in North Dakota, Minnesota and Nebraska were examined for presence of *Cercospora beticola*, the causal agent of Cercospora leaf spot (CLS) of sugar beet. As part of an ongoing nationwide survey, soils were collected from several areas including sugar beet and non sugar beet growing fields. The sugar beet fields were either under beet cultivation or in rotation with other crops. Others fields include fields with no previous history of sugar beet or never been cultivated. The soils were subjected to Enzyme-Linked Immunosorbent Assay (ELISA) and PCR. The samples were subjected to ELISA using pre-adsorption *C. beticola* specific antibodies. For PCR, total DNA was extracted from soil samples using PowerSoil DNA Kit (MO BIO, CA) as per the manufacturer's instructions. Target DNA fragments were amplified using Extract-N-Amp PCR mix (Sigma Aldrich, St Louis MO). The reactions were primed with *C. beticola* specific actin sequence based primers CBACTIN959 L (5′ GTAAGTGCTGCCACAATCAGAC 3′) and CBACTIN959 R (5′ TACCATGACGATGTTTCCGTAG 3′). The amplicons were resolved by electrophoresis in 1% agarose gels. Using ELISA and PCR, we detected *C. beticola* in soil at several locations. Contrary to prevailing views, we also detected *C. beticola* also in field soil which are not under sugar beet cultivation. This survey identified areas even with no history of sugar beet with risk of CLS on susceptible crops. The pathogen might have been from known or yet to be identified alternate hosts.

An epidemic of Septoria canker on *Populus balsamifera* in northern Alberta

J. M. LEBOLDUS (2), P. V. Blenis (2), B. R. Thomas (1)
(1) Alberta-Pacific Forest Industries Inc., Boyle, Alberta, Canada; (2) University of Alberta, Edmonton, Alberta, Canada
Phytopathology 98:S87

Both *Populus deltoides* and *P. balsamifera* are reportedly resistant to Septoria canker caused by *Septoria musiva*. In response to a recent outbreak of Septoria canker on *P. balsamifera* in a northern Alberta plantation, a greenhouse inoculation study was conducted to determine if the outbreak was caused by a single highly virulent isolate. Four clones of putatively resistant *P. balsamifera*, five clones of putatively resistant *P. deltoides*, and one susceptible hybrid poplar clone *P. balsamifera* × *P. deltoides* var. Northwest were inoculated with seven isolates of *S. musiva*. Four of the isolates were from *P. balsamifera* in Alberta and the others were isolated from hybrid poplars in Quebec. Two of the Quebec isolates were the most virulent of 19 isolates evaluted in a previous experiment and the third was the least virulent in that experiment. Preliminary results indicated that *P. deltoides* was more resistant to stem infection than *P. balsamifera* and that Northwest was of intermediate resistance. Disease severity was similar for Alberta and Quebec isolates. In addition to these results, canker incidence and canker age were recorded on a plantation including Northwest and 61 different clones of *P. balsamifera*. Canker incidence was similar for the two species and most cankers were less than 1-year old. In summary, the outbreak of Septoria canker on *P. balsamifera* does not appear to have been caused by a single highly virulent isolate, the early stages of the epidemic appear to be similar on both Northwest and *P. balsamifera*, and contrary to what is assumed in the literature, *P. balsamifera* appears quite susceptible to Septoria canker.

Hydrogen cyanide of *Pseudomonas chlororaphis* O6, kills root knot nematode, *Meloidogyne hapla*

J. LEE (1), K. Ma (2), Y. Kim (1)
(1) Environmental Friendly Agricultural Research Center, Chonnam National University, Gwangju, Korea; (2) Fruit Crop Experiment Station, Chonnam Agricultural Research & Experiment Service, Haenam, Jeonnam
Phytopathology 98:S87

Plant growth promoting rhizobacterium, *Pseudomonas chlororaphis* O6, has strong antifungal activity and produces hydrogen cyanide, protease, and phenazine. This bacterium was studied to kill nematodes under laboratory condition. *P. chlororaphis* O6 effectively kills root knot nematode, *Meloidogyne hapla*, major plant parasitic nematode of kiwi fruit in South Korea. Hydrogen cyanide liquid media inoculation with *P. chlororaphis* O6 resulted in greater efficacy to kill the root knot nematodes compared to King's B liquid media inoculation with the rhizobacteria. In addition, GacS mutant of *P. chlororaphis* O6 which is not producing hydrogen cyanide, presented significantly different to control root knot nematodes compared to wild type. The active concentration of the rhizobacterium was 10^8/cfu and the nematodes knocked down after two hours inoculation with *P. chlororaphis* O6 and most nematodes were killed after three hours inoculation. The results demonstrated that hydrogen cyanide is major role to kill the nematodes and *P. chlororaphis* O6 might have potential value to use as biocontrol agents for control root knot nematode in kiwi fruit. Greenhouse assay with tomato plant will be conducted through naturally infected soils.

Analysis of genomic variation of rice blast resistance gene *Pi-ta* in *Oryza* species

S. LEE (2), Y. Jia (1)
(1) USDA-ARS Dale Bumpers National Rice Research Center; (2) University of Arkansas, Rice Research and Extension Center
Phytopathology 98:S87

The resistance gene *Pi-ta* in rice has been deployed worldwide to prevent the infection by blast pathogen, *Magnaporthe oryzae*. The genomic region

spanning *Pi-ta* in 144 accessions composed of seven *Oryza* species has been sequenced to determine DNA sequence variation of *Pi-ta*. Presently, three significant clades of the *Pi-ta* alleles were found among 144 accessions. Nucleotide differences in the 5' leader and 3' trailer regions were observed to distinguish *Pi-ta* variants. The first significant insertion of 266bp was observed at the translation start codon ATG in 19 accessions composed of *O. Sativa* (red rice and ancient rice cultivars Carolina Gold and Blue rose), *O. rufipogon*, *O. nivara*, *O. barthii*, *O. glaberima* and *O. glumaepatula*. An additional 313bp insertion was observed at 465bp after the translation stop codon TGA in 3' trailer region in six accessions of *O. barthii*, *O. glaberima* and *O. glumaepatula*. Phylogenetic analysis of *Pi-ta* alleles among the 144 accessions revealed four genetic linkage groups distinguishing each *Oryza* species. *Pi-ta* alleles in *O. rufipogon* showed the highest similarity with *O. sativa* (Asian, U.S. cultivars, and weedy red rice U.S. biotypes). The *Pi-ta* alleles in red rice were more similar to both Asian and U.S. cultivars than to wild rice relatives. Results on how these sequence variations may impact *Pi-ta* resistance and lead to a better understanding of the origin of *Pi-ta* will be presented.

Identification and characterization of interacting proteins of the *AVR-Pita* metalloprotease of *Magnaporthe oryzae* using the yeast two-hybrid system

S. LEE (2), X. Wang (3), Y. Jia (1)
(1) USDA-ARS Dale Bumpers National Rice Research Center; (2) University of Arkansas, Rice Research and Extension Center, Stuttgart, AR; (3) Zhejiang University, Hangzhou, P. R. China
Phytopathology 98:S88

The avirulence gene in the pathogen is proposed to promote plant diseases and is also called an effector. The plant resistance gene, on the other hand, detects the effector to trigger resistance. *AVR-Pita* is predicted to encode a metalloprotease and its expression is induced during pathogenesis. However, the product of the resistance gene *Pi-ta* in rice appears to recognize the processed product of AVR-Pita protein-AVR-Pita$_{176}$ produced by *Magnaporthe oryzae* to initiate effective resistance responses. To determine other plant and fungal factors that may interact with *AVR-Pita* in rice, the yeast two-hybrid system was used to identify the AVR-Pita interacting factors. A cDNA library was constructed using pool of mRNAs of Katy (*Pi-ta/Pi-ta*) harvested 6, 12, and 24 hrs after inoculation with a compatible race IE-1K or an incompatible race IB-49. The average insert size of library was 1.7 kb, and the library was found to contain 9 × of the entire rice genes in the genome. In a preliminary screening, a putative zinc finger protein was identified to interact with *AVR-Pita*. This zinc finger protein has been induced by rice blast pathogen and it is likely involved in pathogenesis. Progress in determining whether this zinc finger protein is a target of the AVR-Pita metalloprotease will be presented.

A newly emerging potato disease associated with 16SrIII phytoplasmas in Montana

I. LEE (1), K. D. Bottner (1), M. Sun (2)
(1) Molecular Plant Pathology Lab., USDA-ARS, Beltsville, MD 20705, USA; (2) Potato Lab., Montana State University Extension Service, Bozeman, MT 59717, USA
Phytopathology 98:S88

Potato purple top (PPT) syndrome can be induced by infection with various plant pathogens including phytoplasmas. There are at least five distinct phytoplasma strains belonging to five different phytoplasma groups (16SrI, 16SrII, 16SrVI, 16SrXII, and 16SrXVIII) that were reported to cause purple top and related symptoms in potatoes in North America, Mexico, Russia, and elsewhere. A newly emerging potato disease with extensive yellowish purple discoloration of terminal shoots and leaves similar to PPT syndrome reported previously was observed in some isolated potato fields in Montana in 2007 when an unusual drought occurred in the region. The symptomatic plants exhibited moderate rosette formation and stunting. A phytoplasma was detected in all symptomatic samples and identified to be a new member of 16SrIII group, most closely related to subgroup 16SrIII-F. This phytoplasma strain can be graft-transmitted to and cause shoot proliferation on tomato plants. Pathogenicity tests were carried out by grafting tomato scions onto healthy potato plants. Infected potato plants exhibited mild to moderate symptoms. Potato chips prepared from the infected tubers showed brown discoloration of vascular and pith tissues characteristic of phytoplasmal infection. Most infected tubers, however, produced near normal sprouts and the seedlings exhibited moderate rosette formation and stunting. Some infected plants also exhibited purple top discoloration. This phytoplasma strain appears to cause only mild to moderate symptoms unlike the 16SrVI-A strain that causes severe potato purple top wilt in Washington and Oregon. This is the first report of 16SrIII group phytoplasmas causing diseases in potato.

Occurrence of *Zucchini yellow mosaic virus* and *Cucumber green mottle mosaic virus* on cucumber (*Cucumis sativus* L.) in plastic house in Korea

J. LEE (1), D. Kim (1), Y. Ryu (1), S. Park (1), K. Choi (1), J. Yoon (1), K. Lee (2)
(1) Gyeongbuk Agricultural Technology Administration, Daegu, Korea, 702-708; (2) School of Applied Biosciences, Kyungpook National University, Daegu, Korea 702-701
Phytopathology 98:S88

In Korea, cucumber is high valued cash crop, for it is grown during the winter season in a plastic house. Recently, virus disease spread widely in cucumber growing area and causes severe income loss. To prevent virus disease, occurrence of virus diseases on cucumber were surveyed from 2004 to 2007. Infection rate was 66% with 15~90% of diseased plants per plastic house. The 711 virus infected plants were analyzed by RT-PCR using appropriate detection primer. *Zucchini yellow mosaic virus* (ZYMV) was the most important virus which was detected from over 80% of plants and followed by *Cucumber green mottle mosaic virus* (CGMMV) (56%); *Water melon mosaic virus*-2 was not found. During the early growing stage, ZYMV symptom on leaves frequently first found near the entrance of a plastic house and then spread into the inner area; about 50% of cucumber in a house were infected by ZYMV within 30 days probably by pruning practice. Whereas, CGMMV first found randomly in the plastic house. About 20% of pumpkin plants growing outside of plastic house carried ZYMV, suggesting that it is the main source of ZYMV transmission to the cucumber. ZYMV infected plants showed chlorosis and severe mosaic symptom on leaves and abnormal distortion on fruits. When cucumber plants were infected by ZYMV or CGMMV, marketable yield was reduced as much as 50~70%. Keywords: Cucumber, plastic house, *Zucchini yellow mosaic virus*, *Cucumber green mottle mosaic virus*.

Quantitative trait loci (QTL) associated with bacterial blight and blast resistance in Korean rice populations

S. LEE (5), J. Jeung (2), S. Han (5), D. Ra (5), H. Leung (3), S. Hulbert (4), J. Leach (1)
(1) Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80521; (2) Genetics and Breeding, NICS, RDA, Suwon, Korea; (3) Plant Breeding, Genetics and Biotechnology, IRRI, Philippines; (4) Plant Pathology Dept., Washington State University, Pullman, WA 99164; (5) Plant Pathology Div., NIAST, RDA, Suwon 441-707, Korea
Phytopathology 98:S88

Disease resistance governed by quantitative trait loci (QTL) may provide broad spectrum, durable resistance to rice diseases caused by fungi (rice blast) and bacteria (bacterial blight). QTL analysis (166 genes X 8 restriction enzyme digests) of 164 individuals of a recombinant inbred population and their parents (Milyang23, Gihobyeo) revealed 96 polymorphic genes. Resistance gene homologs (35) and defense response genes (2) from rice, barley and maize were mapped on an existing linkage map consisting of 168 RFLP markers; 205 markers were placed throughout the 12 rice chromosomes with an average distance of 7.46 cM between markers. Based on diseased leaf area, lesion size and lesion length using Korean and Philippine blast fungal isolates and a Korean bacterial blight isolate, 25 resistance QTL were identified on chromosome 1, 3-6, 8-10, and 12. Eleven QTL were associated with 13 R-gene homolog markers on chromosome 1, 5, 8 and 9. Some NB-LRR homologs are associated with more than one disease resistance QTL, e.g., rNB84 on chromosome 1 is associated with four blast resistance QTL. Interestingly, a cluster of resistance gene homologs on chromosome 5 are associated with both blast and bacterial blight resistance. QTL detected on the long arm of chromosomes 1 and 12 were identified by many different blast isolates, consistent with the QTL being involved in broad spectrum resistance.

Development of transformation and RNA-mediated gene silencing systems for functional genomics of *Cochliobolus sativus*

Y. LENG (2), C. Wu (1), J. Rasmussen (2), S. Zhong (2)
(1) Department of Microbiology, University of Hawaii at Manoa, Honolulu, HI, USA; (2) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA
Phytopathology 98:S88

Cochliobolus sativus (anamorph: *Bipolaris sorokiniana*) is an important fungal pathogen of wheat and barley. It causes spot blotch, common root rot and black point on both of the crops. To characterize the genes for pathogenicity and virulence in *C. sativus*, we developed transformation system and are testing RNA-mediated gene silencing for the fungus. A green fluorescent protein (GFP) gene was used for transformation by the polyethylene glycol (PEG)-mediated transformation method. Eleven transformants were obtained from the fungal isolate, ND93-1, and a high level expression of the GFP was observed in mycelia and conidia produced by 8 of the transformants. The *Tox A* gene from *Pyrenophora tritici-repentis*, the tan spot pathogen of wheat, was also used to transform two isolates (ND93-1 and

ND90Pr) of *C. sativus*. Three tranformants from each of the two isolates were generated with Tox A. PCR amplification and inoculation experiments on differential wheat and barley genotypes indicated that Ptr Tox A was expressed in *C. sativus*. A silencing vector was modified from pSlient-1 to adapt to the Gateway Cloning System and is being used to express the hairpin RNA of GFP or Tox A through transformation in attempts to silence the two genes in the transformants. The transformation and gene silencing systems established will be very useful for functional genomics studies in *C. sativus*.

Evaluation of selected soybean genotypes for resistance to *Phakopsora pachyrhizi*

S. LI (1), L. D. Young (1)
(1) USDA-ARS, Crop Genetics and Production Research Unit, Stoneville, MS 38776
Phytopathology 98:S89

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd., is one of the most destructive diseases of soybean (Glycine max (L.) Merr.). ASR has been discovered in United States and has the potential to causes significant yield losses and major economic damage to U.S. soybean production. Breeding for resistance to soybean rust is one of the most effective long-term strategies for controlling ASR. To identify new sources of resistance to domestic ASR isolates, our strategy is to evaluate soybean lines that were previously identified as resistant with foreign isolates. In this study, two sets of plant introductions (PI) were evaluated using an ASR isolate from Mississippi. The first set of PIs contained 10 lines previously identified as resistant in Paraguay and the second set had 17 lines that were selected based on information from Germplasm Resources Information Network (GRIN). Replicated experiments were conducted in growth chambers at the Stoneville Research Quarantine Facility from 2007 to 2008. Soybean line PI567102B was identified as the most resistant line. It had the lowest severity, no sporulation, and the red-brown reaction. Collaborative research is underway to evaluate a segregating population with PI567102B as a parent. Soybean lines having resistant reactions to both U.S. and foreign isolates may be important for developing elite cultivars with broad resistance to ASR.

Effects of host resistance and shading density on the disease severity of hydrangea leaf spot

Y. LI (2), M. T. Windham (1), R. N. Trigiano (1), A. S. Windham (2), S. M. Reed (3), J. M. Spiers (4), T. A. Rinehart (4)
(1) Dept. of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN; (2) Soil, Plant and Pest Center, University of Tennessee, Knoxville, TN; (3) USDA/ARS Floral & Nursery Plants Research Unit, McMinnville, TN; (4) USDA/ARS Thad Cochran Horticultural Research Laboratory, Poplarville, MS
Phytopathology 98:S89

Leaf spot, caused by *Cercospora hydrangeae* Ellis & Everh., is a common disease of bigleaf hydrangea (*Hydrangea macrophylla*) in ornamental nurseries and gardens. Experiments were conducted to determine the effects of cultivar and shading density on the disease severity. Two year-old plants of six bigleaf hydrangea cultivars were purchased from Bell Family Nursery, Aurora, OR and transplanted in field plots. Shading treatments included 0% (full sun), 30%, 60% and 90% shade. Disease indices were assessed for leaf spot using a 0–5 index scale on September 17, 2007. Shading treatments significantly (P = 0.0003) affected the disease indices. In general, increasing shade decreased disease severity of leaf spot. Because of the significant (P = 0.0380) interaction between shading and cultivar treatments, comparisons of disease indices among cultivars were conducted in each shading treatment. The results showed that the disease indices were not significantly different among cultivars for the 60% (P = 0.2828) and 90% (P = 0.4348) shading treatments. However, significant differences in disease indices were detected among cultivars in 30% shading (P = 0.0053) and full-sun (P = 0.0001) treatments. ‘Fasan’, ‘Blue Deckle’ and ‘Lilacina’ had significantly lower disease severities than ‘Miranda’ and ‘Pretty Maiden’, whereas ‘Sister Theresa’ was intermediate in both 30% shading and full sun. The present results provide knowledge in the disease management and resistance screening for hydrangea leaf spot.

PopW of *Ralstonia solanacearum*, a harpin that can induce tobacco resistance to tobacco mosaic virus

J. LI (1), H. Liu (1), J. Guo (1)
(1) Department of Plant Pathology, Nanjing Agricultural University, Nanjing, Jiangsu, China
Phytopathology 98:S89

Harpins, such as HrpN of *Erwinia amylovora*, are extracellular glycine-rich proteins that elicit the hypersensitive reaction (HR). We identified popW of *Ralstonia solanacearum*, which encodes a protein similar to known harpins in characteristics of being acidic, rich in glycine and serine, and lacks cysteine. When infiltrated into plants, PopW induced rapid tissue collapse, which required active plant metabolism. The HR-eliciting activity was heat stable and protease sensitive. Thus, we concluded that PopW is a harpin. It had region homologous to pectate lyases of a unique class, but no pectate lyase activity was detected. This suggested that PopW may be targeted to the plant cell wall, and it was confirmed by subcellular location with the green florescent protein. However, popW mutants retained the wild-type ability to elicit the HR in nonhosts and to cause disease in hosts. Meanwhile, the PopW purified from *E. coli* by heterogeneous expression induced tobacco resistance against tobacco mosaic virus (TMV) by 100%. Expression level of the SAR marker gene PR-1 was obviously up-regulated after 12 hours PopW spraying tobacco leave. It was deduced that plant-signaling molecule salicylic acid (SA) plays an important role in PopW induced tobacco resistance against TMV, so it was the SA-dependent systemic acquired resistance (SAR). PopW was a harpin of *Ralstonia solanacearum* and it provided an attractive tool for the improvement of disease control.

Novel cool-season grass endophytes with unique defensive properties by protoplast fusion

H. LI (1), K. D. Craven (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA
Phytopathology 98:S89

Epichloë and *Neotyphodium* endophytes are biotrophic symbionts that form mutualistic relationships with cool-season grasses. They are known collectively for the production of four distinct alkaloid classes with unique anti-herbivore activities, although no endophyte is known to produce all four. The lolines and peramine are known for their anti-insect properties, while the lolitrems and ergot alkaloids are known for their mammalian toxicity. In order to improve the beneficial effects but lessen toxicity to foraging livestock, we are attempting to create new artificial hybrid endophytes through protoplast fusion. Protoplast fusion offers greater stability of somatic hybrids where heterokaryon stability may be an issue. Alkaloid profiles were estimated via PCR from isolates of *E. festucae*, *E. typhina* and *E. elymi*. Protoplasts isolated from one isolate of *E. festucae* producing lolines and peramine compounds were fused with protoplasts of *E. elymi* and *E. typhina* producing lolines only, but with desirable host compatibilities. The protoplast regeneration rate is approximately 10% of the total number of protoplasts fused. Putative “fusants” were selected based upon multiple microsatellite markers indicative of both “parental” lines. Thus, we have obtained 104 PCR-positive hybrids from protoplast fusion of *E. festucae* and *E. typhina*, and 21 hybrids from the combination between *E. festucae* and *E. elymi*, at a fusion rate of 7.6%–28.6% from polyethylene glycol treatment and 1.9%-5.2% from electrofusion. Stable fusants were purified by successive subcultures and hyphal tip/single spore isolation. The resultant stable fusants will be inoculated into desired cultivar for pathogenicity and host compatibility assessment.

A reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens

R. Li (2), R. MOCK (2), Q. Huang (2), J. Abad (1), J. Hartung (2), G. Kinard (2)
(1) USDA-APHIS, Beltsville, MD, USA; (2) USDA-ARS, Beltsville, MD, USA
Phytopathology 98:S89

Polymerase chain reaction (PCR)-based tests are now widely used to detect many plant pathogens such as viruses, viroids, phytoplasmas and bacteria. Preparation of template samples is laborious and costly when large number of samples and multiple pathogens are tested. A reliable extraction method is described in this report for extraction of total nucleic acids from at least ten plant genera including Ipomoea spp., Malus spp., Prunus spp. and Ribes spp. for subsequent detection of plant pathogens. The method combined a modified CTAB (cetyltrimethylammonium bromide) extraction protocol with a semi-automatic homogenizer (FastPrep® instrument) for rapid sample processing and low potential of cross-contamination. The method was applied to sample preparation for PCR-based detection of thirteen RNA viruses, three DNA viruses, six viroids, two phytoplasmas and two bacterial pathogens from different host plants. The procedure is cost-effective and the qualities of the nucleic acid preparations are at least equal to those prepared by commonly used commercial kits. The efficiency of the procedure permits processing of numerous samples and the use of a single nucleic acid preparation for testing both RNA and DNA genomes by PCR, making this an appealing method for testing multiple pathogens in certification and quarantine programs.

Urediospore germination and infection of *Phakopsora pachyrhizi* on soybean under light

X. LI (1), X. Yang (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 98:S89

Two experiments were conducted to examine effects of light (fluorescent light of color temperature 6500K) on germination and infection of urediospores of soybean rust caused by *Phakopsora pachyrhizi* Sydow. In the first experiment,

urediospore suspension was placed on glass slides and then sealed with parafilm. The slides were then treated with different combinations of dark/light periods at temperature 22–24°C. Irradiance of visible light on the spores was in a range of 1.2–1.5 W/m^2 under the florescent light. Microscopic observations at 2/4/6/8 hours after incubation indicated that the formation of appresoria started at about 4 hours after incubation under either dark or light condition. The spore germination rate and rate of appresoria formation in treatments with light were significantly lower than those without light. In the second experiment, soybean leaves incubated in the dew chamber under the temperature of 22–24°C were inoculated with the urediospores and treated with 0/4/8/12 hours dark periods respectively. Then the leaves were exposed to the same artificial light until 24 hours after the inoculation. After that, leaves were incubated under 25–27°C with diurnal cycle of 12-hour light of. More uredia were produced on the leaves treated with longer dark periods at 12–14 days after inoculation.

Regional predictive modeling and the occurrence of soybean rust caused by *Phakopsora pachyrhizi* in Iowa in 2007

X. LI (1), S. Navi (1), X. Yang (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 98:S90

Soybean rust (*Phakopsora pachyrhizi* Sydow) urediospore dispersal predictions and local rainfall favorability to the disease development forecasts were made during the 2007 growing season using a weather-dispersal-disease coupled model. The spore dispersal forecasts in a medium time range (1–4 weeks) were based on the regional atmospheric circulations predicted by the large-scale meteorological models (MM5). Our forecasts on dispersal of soybean rust spores indicated a large amount of urediospore deposition in western to central Iowa in middle August. On September 25, the first detection was reported in Dallas County in central Iowa. Subsequent field surveys identified the disease in 14 counties throughout Iowa by late October. Most fields had low disease incidence 1–2%. However, in Fremont County of southwestern Iowa the disease incidence was of 15–20% with severity of 2–5%. The disease data from Fremont County suggested the occurrence of multiple infection cycles from local inoculum sources since 6–8 weeks ago, which was consistent with the possible initial infection date in Fremont County as shown by our previous forecasts. Analysis of spore dispersal maps in the midwest over the season together with disease detection data suggests occurrence of soybean rust in Iowa as a result of long-distance jump/s of epicenters or bridging sources. The infested fields in southwestern Iowa appeared to be sources for spore dispersal across Iowa, which was favored by the wetter-than-normal weather in August and September.

A functional genomics approach for characterization of pathogenicity from the necrotrophic phytopathogen *Sclerotinia sclerotiorum*

D. LIBERTI (1), K. F. Dobinson (1)
(1) Agriculture and Agri-Food Canada, London, Ontario, Canada
Phytopathology 98:S90

Sclerotinia sclerotiorum (Lib.) de Bary is among the world's most damaging fungal plant pathogens, with an expansive host range. Its economic importance flags *S. sclerotiorum* as an exemplary model for soilborne necrotrophic plant pathogens. The 2005 release of a draft genome sequence provided an invaluable resource for dissecting the biology of this organism. To exploit this resource, we recently developed an *Agrobacterium tumefaciens*-mediated gene disruption transformation protocol, which efficiently led to recovery of monocaryotic stable mutants. A malate synthase (*mls1*), the key enzyme of the glyoxylate cycle, was chosen as a study case. Preliminary data showed that *mls1* was required for *S. sclerotiorum* pathogencity. In this study we show that *mls1* pathogenicity is dependent on lipid mobilization and fatty acids conversion into oxalate, the major *S. sclerotiorum* pathogenicity factor. In order to better understand the glyoxylate cycle involvement in *S. sclerotiorum* pathogenicity, we generated another glyoxylate cycle deficient mutant by disruption of a homologue of *pth2*, a peroxysomal carnitine acetyltransferase gene, which was recently characterized in *Magnapothe grisea*. It appeared that like *mls1*, *pth2* is essential for fatty acid metabolism. Further characterization of *pth2* disruption mutants and the role of *pth2* in sclerotial development, ascospores production, and pathogenicity will be discussed.

Functional characterization of necrosis and ethylene-inducing like proteins (NLPs) from a necrotrophic fungus *Sclerotinia sclerotiorum*

D. LIBERTI (1), D. Qutob (1), M. Gijzen (1), K. F. Dobinson (1)
(1) Agriculture and Agri-Food Canada, London, Ontario, Canada
Phytopathology 98:S90

Necrosis and ethylene-inducing like proteins (NLPs) constitute a growing family of secreted proteins, produced by a variety of eukaryotic and prokaryotic microorganisms, with a common ability to elicit a cell death response in dicotyledonous plants. We found two distinct *NLP* genes, *NLP$_{Ss}$1* and *NLP$_{Ss}$2*, in the draft genome sequence of a necrotrophic plant pathogenic fungus *Sclerotinia sclerotiorum*. These genes are highly expressed during the necrotrophic phase of disease development, presumably to accelerate host cell death. We developed a strategy for *NLP$_{Ss}$1* and *NLP$_{Ss}$2* functional characterization through generation of knock-out (KO) mutants and analysis of mutant phenotypes. Full length *NLP$_{Ss}$1* and *NLP$_{Ss}$2* were individually cloned into a binary vector and disrupted by transposon mutagenesis. *Agrobacterium tumefaciens*-mediated transformation was applied to integrate *NLP$_{Ss}$1* or *NLP$_{Ss}$2* KO cassettes into the *S. sclerotiorum* genome. Resulting transformants were screened by PCR amplification and DNA blot hybridization to identify those in which wild-type genes have been replaced by disrupted copies. For *NLP$_{Ss}$1* disruption all transformants recovered contained a single-copy targeted gene replacement. In contrast, *NLP$_{Ss}$2* transformants contained only ectopic insertions of the disrupted gene. These data suggest that *NLP$_{Ss}$2* may be indispensable for viability, and/or that the *NLP$_{Ss}$2* locus has a very low recombination frequency. In addition, we demonstrated meiotic and mitotic stability for three *NLP$_{Ss}$1* mutants by producing single-ascospore isolates in which no wild-type transcript accumulation was observed. The necrotrophic life style of these mutants and the role of NLPs in pathogenicity will be discussed.

Infectious clones of *Alternanthera mosaic virus* inducing distinct symptoms aid identification of symptom determinants

H. Lim (2), A. Vaira (1), M. D. Reinsel (2), H. Bae (2), J. HAMMOND (3)
(1) USDA-ARS - and - CNR, IVV, Torino, Italy; (2) USDA-ARS, Beltsville, MD, USA; (3) USDA-ARS, USNA, FNPRU, Beltsville, MD, USA
Phytopathology 98:S90

The potexvirus *Alternanthera mosaic virus* (AltMV) naturally infects several ornamental plants; AltMV-SP was isolated from *Phlox stolonifera* and maintained in *Nicotiana benthamiana*. The 5′ and 3′ regions of the genome were amplified by PCR; four infectious clones were created from AltMV-SP by ligation of two independent clones of each of the 5′ half [RdRp(3 or 4)] and 3′ half [Pol+TGB+CP(1 or 7)] of the genome, and designated 3-1, 3-7, 4-1, and 4-7. Each clone produces distinct symptoms; 3-7 and 4-7 induce severe symptoms, 3-1 induces mild symptoms, and 4-1 does not induce distinct symptoms. Symptom severity was determined by the 3′ half of the genome; sequence analysis identified amino acid differences K(1124)R and R(1125)K in Pol domain, and P(88)L in TGB1. When amino acids K(1124) and R(1125) in mild clones were substituted by R and K, necrosis was observed within 30 days. Substitution of P(88) by L in mild clones enhanced viral RNA accumulation, and induced necrosis within 50 dpi. Substitution of all three amino acids (Pol+TGB1) into either mild strain induced severe necrosis in 10 dpi. Each protein was analyzed using Agroinfiltration and the yeast two hybrid system. Substitution of P(88) to L in TGB1 enhanced gene silencing suppressor activity, but no difference was observed in TGB1 self interaction or subcellular localization.

The blast resistance gene Pi37 encodes an NBS-LRR protein and is a member of a resistance gene cluster on rice chromosome 1

F. LIN (1), S. Chen (1), Z. Que (1), L. Wang (1), X. Liu (1), Q. Pan (1)
(1) College of Natural Resources & Environment, South China Agricultural University, Guangzhou 510642, China
Phytopathology 98:S90

The resistance (R) gene Pi37, present in the rice cultivar St. No. 1, was isolated by an in silico map-based cloning procedure. The equivalent genetic region in Nipponbare contains four NBS-LRR type loci. These four candidates for Pi37 (Pi37-1, -2, -3 and -4) were amplified separately from St. No. 1 via long-range PCR, and cloned into a binary vector. Each construct was individually transformed into the highly blast susceptible cultivar Q1063. The subsequent complementation analysis revealed Pi37-3 to be the functional gene, while -1, -2 and -4 are probably pseudogenes. Pi37 encodes a 1290 peptide NBS-LRR product, and the presence of substitutions at two sites in the NBS region (V239A and I247M) is associated with the resistance phenotype. Semi-quantitative expression analysis showed that in St. No. 1, Pi37 was constitutively expressed and only slightly induced by blast infection. Transient expression experiments indicated that the Pi37 product is restricted to the cytoplasm. Pi37-3 is thought to have evolved recently from -2, which in turn was derived from an ancestral -1 sequence. Pi37-4 is likely the most recently evolved member of the cluster, and probably represents a duplication of -3. The four Pi37 paralogs are more closely related to maize rp1 than to any of the currently isolated rice blast R genes Pita, Pib, Pi9, Pi2, Piz-t and Pi36.

***Pseudomonas syringae* – gene characterization and genome mapping for the next generation**

M. LINDEBERG (1), A. Collmer (1), D. J. Schneider (2)
(1) Cornell University, Ithaca, NY, USA; (2) USDA-ARS, Ithaca, NY, USA
Phytopathology 98:S90

Since publication of the complete genome sequence for *P. syringae* pv. *tomato* DC3000, diverse analyses have significantly enhanced our under-

standing of *P. syringae* genome structure, evolution, and genetic repertoire. Major sources of new information include ongoing experimental characterization of DC3000 genes and gene orthologs from other strains and species, identification of new genes based on improved computational predictions, and confirmation of expression based on transcript analysis and proteomics data. Identification of extragenic features including repetitive sequences, insertion sequence scars, and gene fragments provide additional insight on the evolutionary events that have shaped genome structure. Distribution of various features relative to overall P. syringae genome organization was analyzed by mapping core and variable regions on the basis of deviations in sequence composition and gaps in syntenic alignment relative to genome sequences of *P. s.* pv. *syringae* B728a and *P. s.* pv. *phaseolicola* 1448A. These analyses resulted in identification of 121 variable regions, accounting for over 35% of the genome. Variable regions are enriched for particular classes of virulence factors including plant-active toxins and members of the HrpL regulon, as well as mobile elements and genes of unknown function. These last, associated as they are with regions of likely horizontal transfer, are of particular interest as a potential reservoir of novel host-interaction determinants. Ongoing characterization of the locations, expression, and phenotypic properties of genes in all three *P. syringae* genomes is producing major updates in the *P. syringae* genome annotations. Updates made to the *P. syringae* genome files at Genbank, Gene Ontology Annotation of confirmed virulence factors, and files for viewing predicted variable regions and other newly identified features using the Artemis genome viewer can be found at the Pseudomonas-Plant Interaction website (http://www.pseudomonas-syringae.org/). Graphical representations of genome comparison between DC3000 and other sequenced bacterial genomes can also be found there.

CAPS markers in an eIF4E gene are linked to *Zucchini yellow mosaic virus* resistant locus in watermelon

K. LING (3), K. Harris (3), J. D. Meyer (1), A. Levi (3), N. Guner (2), T. C. Wehner (2), M. J. Havey (4)
(1) Department of Horticulture, University of Wisconsin, Madison, WI; (2) Department of Horticulutral Science, North Carolina State University, Raleigh, NC; (3) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC; (4) USDA-ARS, Vegetable Crops Unit, Madison, WI
Phytopathology 98:S91

Zucchini yellow mosaic virus (ZYMV) is an economically important virus infecting cucurbits worldwide. Genetic sources of resistance to ZYMV have been identified in wild watermelon germplasm where a single recessive gene (*zym*) confers ZYMV resistance. Marker-assisted selection (MAS) would greatly facilitate the breeding process. Currently, DNA markers are not available for MAS. The objective of this study was to identify molecular markers linked to the ZYMV resistant locus in watermelon. Previous studies by others have shown that genes encoding the eukaryotic initiation factors (eIF) 4E and (iso)4E are associated with the recessively inherited resistance to potyviruses in a number of plant species. Using a candidate gene approach, eIF4E and eIF(iso)4E gene sequences in watermelon were PCR-amplified from the ZYMV-resistant PI 595203 and the ZYMV-susceptible cv. New Hampshire Midget. Single nucleotide polymorphisms (SNP) were identified in the eIF4E sequence between the resistant and susceptible parental genotypes. Co-dominant Cleaved Amplified Polymorphic Sequence (CAPS) markers were created using the identified SNPs in the eIF4E genes. These CAPS markers were evaluated for their linkage to the ZYMV resistant gene in F_2 and BC_1 populations. Although a marker in the eIF(iso)4E gene was not linked, markers in eIF4E were shown to have a close linkage to the ZYMV resistant locus in watermelon.

Development of an improved real-time PCR system for broad-spectrum detection of diverse *Didymella bryoniae* genotypes

K. LING (4), W. P. Wechter (4), B. M. Somai (3), R. R. Walcott (2), A. P. Keinath (1)
(1) Coastal Research &Education Center, Clemson University, Charleston, SC; (2) Department of Plant Pathology, The University of Georgia, Athens, GA; (3) Nelson Mandela Metropolitan University, South Africa; (4) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC
Phytopathology 98:S91

Gummy stem blight (GSB) is a major disease of cucurbit crops (e.g., cantaloupe, cucumber and watermelon) in the southern United States. *Didymella bryoniae* (anamorph *Phoma cucurbitacearum*), the causal agent of GSB, is often isolated from infected tissues together with other Phoma spp. RAPD profiles and PCR primers have been used to differentiate two genotypes (RG I and RG II) of *D. bryoniae* from each other and from non-pathogenic *Phoma* spp. RG I isolates are more virulent on cucurbits and widespread in major cucurbit growing areas in the world. RG II isolates are less virulent with limited geographical distribution. Existence of different genotypes of *D. bryoniae* with various levels of virulence in field populations makes the use of sequence-based detection methods (e.g., real-time PCR) more challenging. The objectives of this study were to identify a conserved genome sequence derived from RAPD fragments from RG I and RG II isolates and to develop a real-time PCR system with a broad-spectrum reaction to *D. bryoniae* isolates. Three previously unpublished *D. bryoniae* specific primer sets (namely, 17-mer, 21-mer and 25-mer) derived from RAPD products were used in PCR. A conserved sequence region common to both genotypes of *D. bryoniae* was identified in PCR products generated from the 17-mer primer set. New primers and probe were designed and used in real-time PCR. This newly developed real-time PCR system detected a worldwide collection of *D. bryoniae* isolates (including 108 RG I and 20 RG II) with no cross reaction to 13 *Phoma* spp. or to *Colletotrichum*, *Alternaria*, and *Fusarium*.

Development of a multiplex real-time PCR assay for the simultaneous detection of three seedborne pathogen types in cucurbits

K. LING (3), W. P. Wechter (3), R. R. Walcott (2), A. P. Keinath (1)
(1) Coastal Research & Education Center, Clemson University, Charleston, SC 29414; (2) Department of Plant Pathology, University of Georgia, Athens, GA; (3) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC
Phytopathology 98:S91

Cucurbits (e.g., watermelon, melon, cucumber, squash, and pumpkin) are important crops in the U.S. Cucurbit diseases incited by seed-borne pathogens, such as bacterial fruit blotch [*Acidovorax avenae* subsp. *citrulli* (AAC)], gummy stem blight [*Didymella bryoniae* (DB)], and squash mosaic [*Squash mosaic virus* (SqMV)] are especially difficult to control. Effective management of these diseases is often through the use of certified pathogen-free seed lots. Current individual seed health testing methods (e.g., seedling grow-out, culturing or ELISA) are cumbersome and expensive. The objective of this study was to develop a single molecular technique (e.g., real-time PCR) for the simultaneous detection of several pathogen types (viral, bacterial and fungal) in cucurbit seeds. Previously, we developed a real-time PCR system for two pathogens (AAC and DB). Thus, in this study, we focused our efforts in developing a real-time reverse-transcriptase-PCR system for SqMV detection and evaluating the optimum conditions in the multiplex system. To maximize the broad-spectrum detection of various SqMV isolates, a conserved genome sequence region was identified through alignment of multiple SqMV sequences in GenBank. Primers and probe were designed based on the consensus sequence and shown to yield a high signal value in the simplex system prior to multiplexing. Converting the viral RNA to cDNA stabilized the templates for subsequent PCR amplification and allowed for simultaneous detection of three pathogen types in a single reaction. The multiplex real-time PCR detection system was capable of detecting three different pathogens in mixtures of infested seeds.

Quantifying the temporal and spatial spread of *Pantoea stewartii* in sweet corn

L. LIU (1), F. W. Nutter Jr. (1)
(1) Iowa State University
Phytopathology 98:S91

Stewart's disease, caused by *Pantoea stewartii*, can cause severe economic damage to seed and sweet corn enterprises due to mandatory phytosanitary regulations and/or direct yield losses. To date, there is little or no quantitative data on the temporal and spatial spread of this pathogen. Therefore, field experiments were conducted in 2007 in Ames, IA. Seed treated with insecticide (Cruiser) and seed not treated with insecticide were planted in plots 6 rows wide, with 25 corn plants per row. The center six corn plants (variety "Jubilee") in each plot were inoculated with a double-marked strain of *Pantoea stewartii* (rifampicin-naldixic acid resistant) isolate. To test if the corn plants within plots were infected with the marked strain of *P. stewartii*, feeding scars caused by the vectors (corn flea beetles) were sampled from each plot every 6–10 days, beginning 37 days after inoculation, and continuing until 16 August. The marked strain of *P. stewartii* was first detected on 13 July and the incidence of *P. stewartii*-infected plants was zero in seed-treated plots compared to 0 to 2.8% in non-treated plots. The onset of *P. stewartii* epidemics (time to reach 5%) was earlier for the nontreated plots than for treated plots. By the end of the season, mean incidence for treated plots was 1.6 ± 0.011% and mean incidence within non-treated plots was 3.5 ± 0.025%. The logistic model best fit the temporal pathogen progress data, and the mean rates of pathogen increase over time in nontreated plots were significantly faster compare to rates in treated plots (0.082 logits per day v.s. 0.069 logits per day). Spatial patterns were random for all plots for all sampling times.

Identification of quantitative trait loci (QTLs) responsible for sheath blight resistance in rice using recombinant inbred line population of LemontÍJasmine 85

G. LIU (4), Y. Jia (3), F. J. Correa-Victoria (1), A. McClung (3), J. C. Correll (2)
(1) CIAT; (2) Department of Plant Pathology, University of Arkansas, Fayetteville, AR; (3) USDA-ARS Dale Bumpers National Rice Research Center; (4) University of Arkanas, Rice Research and Extension Center, Stuttgart, AR
Phytopathology 98:S92

Rice sheath blight (RSB) caused by the soil borne pathogen *Rhizoctonia solani*, is one of the most destructive diseases of rice, causing severe losses in rice yield and quality annually. Major gene (s) governing the resistance to RSB have not been found in cultivated rice worldwide. However, rice cultivars with different resistance reactions to RSB have been well documented. In this study, the disease reactions of 250 F5 recombinant inbred lines (RILs) of the cross of LemontÍJasmine 85 population to RSB were evaluated in greenhouses using both micro-chamber and mist chamber methods. A genetic linkage map was constructed with 199 evenly distributed simple sequence repeat (SSR) markers. Eight and nine quantitative trait loci (QTLs) responsible to RSB resistance were identified using phenotypic data from micro-chamber and mist chamber methods, respectively. More chromosomes harboring QTLs were identified using the mist chamber (Chromosomes 1, 2, 3, 5, 6, 9, 10 and 12) than the micro-chamber method (Chromosome 1, 3, 5, 6 and 9). Three QTLs on Chromosomes 1, 5 and 9 were consistently identified using both methods. One major QTL associated with RM245 was determined to contribute 18.2% of phenotypic variation using the micro-chamber and 22.8% in mist chamber method. Inoculated field trials of these RILs to RSB will be conducted in three locations (Arkansas, Louisiana and Texas) to confirm the importance of these QTLs under field conditions.

Quantifying and comparing the aggressiveness of *Pantoea stewartii* isolates under different temperatures

L. LIU (1), C. C. Block (1), F. W. Nutter Jr. (1)
(1) Iowa State University
Phytopathology 98:S92

Stewart's disease, caused by *Pantoea stewartii*, can cause severe economic damage to seed and sweet corn crops due to phytosanitary regulations that prevent the export of grain, as well as direct reductions in yield. To date, there is no quantitative data on the aggressiveness of isolates of *P. stewartii* on corn leaves. To quantify and compare the aggressiveness of three *P. stewartii* isolates, growth chamber experiments were conducted using the sweet corn variety "Jubilee". Plants at the V7 growth stage were inoculated with isolates (*Pantoea stewartii*); these were: Rif 9A, a rifampicin-naldixic acid resistant isolate; and ES9211 or ES 9245 (both wild types). This was done by inoculating single leaves with 2 of the 3 isolates; one on each side of the leaf midrib. There were 24 plants/ replication, and experiments were performed 2 times for each of 5 temperature regimes (constant temperatures of 21°C, 24°C, 27°C, 30°C, and 33°C). Lesion expansion (both acropetaly and basipetaly) were measured beginning on the day that lesions were first visible, and measurements continued at 24-hour intervals until no further lesion expansion was possible (leaves were 100% senesced). Results from lesion expansion experiments to date have shown that rates of lesion expansion averaged over *P. stewartii* isolates was fastest at 27°C and that doubling time for lesions to double in size were 37.7, 38.8 and 44.8 hours at 27°C, 30°C, and 33°C, respectively.

Burkholderia communities in soils with long-term tillage, no-tillage and successional systems

B. LIU (1), F. Louws (1)
(1) Department of Plant Pathology, North Carolina State University
Phytopathology 98:S92

Burkholderia spp. contribute to diverse functions, such as plant growth promoting and biological control benefits. *Burkholderia* spp. are also pathogens of plants and humans. The diversity, dynamics and identity of species in farming systems is an important area of research. *Burkholderia* populations were characterized in soils with long-term tillage (CT), no-tillage (NT) and successional (SC) systems using a most probable number (MPN) microtiter plate assay based on 21 sampling points in each system. MPN assays demonstrated that *Burkholderia* populations were significantly higher in SC soils compared to CT and NT soils. SC sample sites were dominated with successional native species whereas CT and NT were planted to soybeans at the time of sampling. Blast searches and phylogenetic analysis based on the sequences of the 16S rRNA demonstrated different soil management practices modified *Burkholderia* species composition. The soils with CT consisted of uncultured *Burkholderia* species, the dominant species in this system, *B. cepacia*, *B. glathei*, *B. caribensis*, and *B. terrae*. The soils with NT consisted of uncultured *Burkholderia* species, *B. cepacia*, *B. ambifaria*, *B. cenocepacia*, *B. anthina*, *B. caribensis* and *B. terrae*, with an even species distribution. The SC soils consisted of uncultured *Burkholderia* species, *B. caribensis*, *B. terrae*, and *B. ambifaria*, with *B. caribensis*, *B. terrae*, and uncultured *Burkholderia* species as the dominate species. In addition, denaturing gradient gel electrophoresis is in progress to characterize *Burkholderia* species diversity. This research demonstrated that the soil management practices affected the quantity and composition of *Burkholderia* spp.

Characterization of *Pythium* communities in soils from conventional tillage, no-tillage and successional systems

B. LIU (1), F. Louws (1)
(1) Department of Plant Pathology, NCSU
Phytopathology 98:S92

Multiple species of *Pythium* are responsible for causing seed and seedling diseases of agronomic crops. Characterization of soil *Pythium* communities is important for understanding *Pythium* biology and the management of damping-off. *Pythium* populations were characterized in soils with long-term conventional tillage (CT), no-tillage (NT) and successional growth (SC) using dilution plating and colony counts. Analysis of 21 samples using a grid sampling design demonstrated *Pythium* populations were significantly higher (P = 0.05) in SC plots (18.5 cfu/g dry weight soil) than NT levels (11.4 cfu/gdw) with CT numbers the lowest (7.3 cfu/gdw). SC sample sites were dominated with successional native species whereas CT and NT were planted to soybeans at the time of sampling. Individual species were identified using BLAST search of the internal transcribed spacer 1 (ITS1) of the representative isolates (n = 21/system). The soils with CT consisted of *P. spinosum*, *P. irregulare* and *P. ultimum*, and the soils with NT consisted of *P. spinosum*, *P. irregulare* and *P. attrantheridium*, respectively. *P. spinosum* was the dominant species in CT and NT systems. The SC soils consisted of *P. spinosum*, *P. irregulare* and *P. attrantheridium*, with *P. irregulare* and *P. attrantheridium* as the dominant species. In addition, denaturing gradient gel electrophoresis is in progress to characterize *Pythium* species diversity. This research demonstrated that the soil management practices affected the quantity and composition of *Pythium* spp.

Inhibitory activity of the extracts of *Macleaya cordata*, *Reynoutria japonica* and *Scutellaria baicalensis* on plant pathogens

H. Liu (1), L. ZHOU (1)
(1) China Agricultural University
Phytopathology 98:S92

Crude extracts of *Macleaya cordata*, *Reynoutria japonica* and *Scutellaria baicalensis* were studied for their inhibitory activity against three plant bacterial pathogens (i.e. *Agrobaterium tumefaciens*, *Pseudomonas lachrymans* and *Xanthomonas vesicatoria*) and six plant fungal pathogens (i.e. *Alternaria solani*, *Rhizoctonia solani*, *R. cerealis*, *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *vasinfectum*). All the plant extracts showed strong activity on plant pathogens. Among them, *R. japonica* extract showed the strongest antibacterial activity on *A. tumefaciens* and *X. vesicatoria*. The extract from *M. cordata* showed the strongest inhibitory activity on the test fungi that the median effective inhibitory concentration (IC50) values were between 0.04 and 0.76 mg/ml. The inhibitory effects of plant extracts in this study indicate the possible application as antimicrobial materials in agriculture especially for some microorganisms which evolved a resistant mechanism to some antimicrobials such as *Alternaria solani* with its resistance to carbendazim.

The modes of action of *Bacillus* sp. C06 in controlling peach brown rot caused by *Monilinia fructicola*

W. LIU (1), X. Li (1), T. Zhou (1)
(1) Agriculture and Agri-Food Canada, Guelph, Ontario, Canada
Phytopathology 98:S92

Postharvest brown rot caused by *Monilinia* spp. and low-temperature damage in the storage are the main factors limiting the fresh stone fruits supply worldwide. *Bacillus* sp. strain C06 has been identified as an effective biocontrol agent for controlling postharvest brown rot of stone fruits under ambient conditions. In this study, possible modes of action of C06 were proposed and tested. We determined the control efficiency of: (1) cell-free culture filtrate (CFF) and autoclaved CFF (CFFA); (2) an antifungal compound PBAC06 isolated from C06; (3) CFF and CFFA to peaches in different ripening stages. Peaches were sprayed with CFF and CFFA. PBAC06 was tested using wounded peach fruits. Treated peaches were incubated at 23°C. After incubation for 6 days, the treatments with CFF and CFFA reduced brown rot incidence by 91% and 76% respectively, as compared to the control. PBAC06 reduced brown rot by 40% 7 days after the treatment. CFF provided a similar brown rot control on both unripe and ripe fruit (by 97.0% and 96.6%, respectively) after 5 days of the treatment. However, CFFA reduced fruit decay by only 49.2% on unripe peaches,

relatively lower than that of 60.6% on the ripe peaches. The results indicated that antibiosis played a role in this biocontrol system. However, although autoclave was able to abolish the inhibition activity of CFFA against spore germination of M. fructicola, CFFA still reduced brown rot incidence. This implied that other mode of action, possibly inducing resistance, was also involved in this biocontrol system. The different reactions of peaches in different ripening stages to CFFA provided further evidence. Therefore, effective biocontrol of brown rot by C06 may relay on both antibiosis and induced resistance. This work contributes to a better understanding of a biological control system in a postharvest disease.

Investigating signaling components required for *RB*-mediated potato late blight resistance response by RNA interference in Agroinfiltrated leaves of *Nicotiana benthaminana*

Z. LIU (1), D. Halterman (2)
(1) Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, USA; (2) USDA/ARS Vegetable Crops Research, Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706
Phytopathology 98:S93

Late blight, caused by the oomycete pathogen *Phytophthora infestan*s, continues to be one of the most devastating diseases of potato. Although great efforts have been made in selection and breeding for resistance, many current potato cultivars remain susceptible. A key long-term management strategy for combating potato diseases is to develop cultivars with high levels of resistance through identification and integration of major resistance (*R*) genes. The *RB* gene, cloned from the Mexican diploid potato species *Solanum bulbocastanum*, confers broad-spectrum resistance to potato late blight. All transgenic potato lines containing a single *RB* gene exhibit strong foliar resistance to all known races of *P. infestans*. *RB* encodes a protein with coiled-coil nucleotide binding site leucine-rich repeat (CC-NB-LRR) motifs. RB recognizes the *P. infestan*s avirulence gene product ipiO1 (an in-planta induced gene product) in potato and *Nicotiana benthamiana*, initiating the hypersensitive response (HR). Several genes are required for the activation of resistance mediated by NB-LRR R proteins against different pathogen classes. To investigate whether HR mediated by RB requires these accessory components, we silenced previously identified genes in *RB*-containing *Nicotiana benthamiana* plants using a RNA interference-based silencing approach followed by Agroinfiltration of ipiO1. Silencing results revealed that RB activation by ipiO1 is dependent on some accessory components but not others. The RNA interference combined with Agroinfiltration is likely to be a effective approach for screening signaling components required for of *RB*-mediated late blight resistance response.

Detection and differentiation of Potato Cyst Nematode (PCN) and morphologically similar species with the NanoChip® technology

Z. LIU (1), M. K. Nakhla (1), L. Levy (1)
(1) USDA
Phytopathology 98:S93

PCN, *Globodera pallida*, is a quarantine plant pathogen in the United States and differentiation of PCN from morphologically similar species, *G. rostochiensis* and *G. tabacum*, is often difficult. The present molecular diagnosis uses PCR followed by RFLP analysis, as well as a newly developed qPCR for each species. We have developed an alternative method using the NanoChip® electronic microarray to identify these species. The method utilizes DNA probes that hybridize to complementary sequences actively deposited on the chip to recognize matching targets. We amplified three rDNA fragments (220bp, 295bp and 751bp, respectively), each tagged with a biotin molecule, from each species in a single multiplex PCR reaction. The resultant amplicons were tested with a set of probes displaying by two different dye-tagged universal reporters. Here, we report the finding of a panel of nine probes for the detection and differentiation of these three species. They include detection probes for all three species (D2, D5 and D6) and two specific probes for each species (P1 and P3 for *G. pallida*, R1 and R3 for *G. rostochiensis*, and T2 and T3 for *G. tabacum*). A single NanoChip® assay performed detection and differentiation of a species or differentiation of two species simultaneously by combining a detection probe and a species-specific probe or two different species-specific probes in a single test. The technology may provide another flexible and accurate platform for rapid PCN diagnostics.

Towards identification of the *Rhg4* gene for resistance to the soybean cyst nematode

X. LIU (2), S. Liu (1), K. Meksem (1), M. Mitchum (2)
(1) Southern Illinois University; (2) University of Missouri
Phytopathology 98:S93

Soybean cyst nematode (SCN) causes nearly $1 billion worth of soybean yield losses annually in the U.S. The *Rhg4* locus on linkage group A2 plays an important role in SCN resistance; however, the gene for resistance has not been cloned and the molecular mechanism by which plant cells perceive and transduce external signals from cyst nematodes during an incompatible interaction have yet to be elucidated. A candidate *Rhg4* gene that codes for a leucine-rich repeat receptor-like kinase protein (LRR-RLK) was cloned by map-based cloning. LRR-RLKs belong to a resistance gene family that includes the *Xa21* gene for bacterial resistance. To confirm the function of the LRR-RLK candidate gene in SCN resistance, a large collection of soybean TILLING mutants was screened. A nonsense mutant was identified but the SCN resistance phenotype of the mutant was not altered. EcoTILLING and haplotyping studies further confirmed that this gene does not correlate with SCN resistance and ruled out the possibility of functional redundancy by a second copy of the LRR-RLK gene. Moreover, the SCN susceptible phenotype of a recombinant inbred line carrying the *Rhg4* susceptible allele was not complemented by the candidate LRR-RLK gene. Further fine mapping of the *Rhg4* locus has identified additional candidate genes for TILLING and complementation studies. Cloning of the *Rhg4* resistance gene will provide a much needed understanding of the molecular basis of soybean resistance to SCN.

Molecular cloning of *AvrHar* from *Pyrenophora teres* f. *teres*

Z. LIU (1), J. D. Faris (2), M. C. Edwards (2), T. L. Friesen (2)
(1) Department of North Dakota State University, Fargo, ND, USA; (2) USDA-ARS Cereal Crops Research Unit, Northern Crop Science Lab, Fargo, ND, USA
Phytopathology 98:S93

Barley net blotch, caused by the ascomycete *Pyrenophora teres*, is a worldwide and economically important fungal disease of barley. The genetics of barley and *P. teres* interaction has been well studied and shown to contain a gene-for-gene relationship. Here, we make an attempt to clone *AvrHar*, the first Avr gene identified and mapped in *P. teres*, using a map-based approach. Previously we showed AvrHar was linked to an AFLP marker by 2.2 cM in a 178-progeny fungal mapping population. Starting from this AFLP marker, we have successfully constructed a 250 kb physical contig toward AvrHar using a 20X BAC library from one parental isolate and a 6X fosmid library from the other parental isolate. Within this contig, the first 120 kb has 31 predicted genes and is a highly gene-rich region. The remaining 130 kb only contains 2 predicted genes and is rich in retrotransposon and other AT-rich sequences. A PCR marker located at the end of the gene-rich region has been shown to co-segregate with *AvrHar*, but no additional genetic recombination has been found within the repetitive region. Another step of chromosome walking is underway to identify the BAC that could span *AvrHar*. Interestingly, some of the repeat sequence has similarity to that surrounding *AvrLm1*, an Avr gene in *Leptosphaeria maculans*. The gene-rich region, but not the repetitive region, was shown to have high co-linearity with *P. tritici-repentis*, a related pathogen that causes tan spot of wheat.

Rapid detection of *Pythium sulcatum* and *P. violae* in soil and cavity spot lesions of carrots

S. LIVINGSTON (1), M. Davis (1)
(1) UC Davis
Phytopathology 98:S93

Cavity spot of carrot, caused by *Pythium sulcatum* and *P. violae*, is serious disease of carrots grown in the San Joaquin Valley of California. Disease loss is presumably correlated with soil populations of *Pythium* species but enumeration of specific species has been difficult using traditional methods. A rapid and accurate method to quantify *P. sulcatum* and *P. violae* would help growers develop suitable disease management strategies. Samples of carrots from a severely affected field were used to compare direct isolation of the causal agents to detection by PCR-based methods. Direct isolation onto a semi-selective medium yielded *Pythium* spp. from only 8% of the carrots. DNA from diluted freeze-dried samples of carrots or from infested soil was used as a template for PCR and nested PCR. *Pythium* spp. was detected by direct PCR of the freeze-dried carrots in 12% of the samples, whereas *Pythium* was detected in 28% of the samples using nested PCR. Individual *Pythium* species were identifiable in the nested PCR procedure employing species specific primers. Although direct PCR amplification of soil-extracted DNA was usually unsuccessful, DNA of both *P. violae* and for *P. sulcatum* was recovered in 20% of soil samples using nested PCR. These results will be used to develop a risk assessment strategy for cavity spot.

Potential for the use of silicon to alleviate disease stresses in floricultural crop production

J. C. LOCKE (1), M. Omer (1), J. Frantz (1), A. Widrig (1), W. Zellner (2), S. Leisner (2), C. Krause (1)
(1) USDA-ARS-ATRU, Toledo, OH; (2) University of Toledo, Department of Biological Sciences
Phytopathology 98:S93

Although there are a few reports of the beneficial use of supplemental silicon on floricultural crops, this potentially useful management strategy has not

been developed in commercial practice. The extent of silicon uptake and accumulation has been evaluated in over 500 plant species, but few of these species are commonly used as floricultural crops. We report here our survey of over 30 commonly grown floricultural crops which indicates that uptake and accumulation, to at least 0.5% of dried weight, occurs in only a limited number of crops (zinnia, verbena, and sunflower). We also report the effect of supplemental silicon against a variety of pathogen-induced stresses which resulted in varying outcomes. In controlled inoculation trials, powdery mildew development was delayed in silicon treated zinnias and lesion size was restricted. Root rot, caused by Pythium ultimum, was not significantly influenced by supplemental silicon fertigation of either vinca or New Guinea Impatiens. Amendment of the hydroponic nuturent solution with silicon reduced the distribution and delayed the spread of Tobacco ringspot nepovirus (TRSV) in tobacco plants. However, supplemental silicon appeared to increase the number of symptomatic Arabidopsis plants when inoculated with TRSV. These results indicate that there is potential to utilize silicon in management practices to alleviate certain plant disease stresses, but it does not appear to be of a universal benefit.

Further characterization of the toti-like virus causing yellow leafspot of spiraea, and its occurrence in diseased aspen in Minnesota
B. E. LOCKHART (1)
(1) University of Minnesota
Phytopathology 98:S94

Spiraea yellow leafspot sphereical virus (SYLSSV), an aphid-transmitted toti-like virus was first reported from Minnesota in 2002. Recent sequence analysis revealed that the monopartite 7 kb dsRNA genome of SYLSSV contains three ORFs capable of encoding proteins of 34, 35 and 115 kDa respectively. The only significant amino acid sequence identities found (24–34%) were to regions of the gag-pol fusion protein of the totivirus *Giardia lamblia virus*. In 2007 SYLSSV was identified in Minnesota in quaking aspen (*Populus tremuloides*) showing symptoms of severe mosaic and leaf deformation.

Condensed nucleoprotein helices containing circular ssDNA may represent a novel type of plant virus
B. E. LOCKHART (1)
(1) University of Minnesota
Phytopathology 98:S94

Virus-like particles associated with leaf yellowing and leaf distortion in *Diffenbachia* (dumbcane) and *Spathiphyllum* (peace lily) (Aracae) consisted of narrow (6 nm) nucleoprotein filaments that formed condensed rod-shaped helices measuring 20 × 250 nm. Electrophorteic analyses and nuclease digestion assays indicated that these virus-like structures contain a major structural protein of approx. 44 kDa and a circular ssDNA approx. 10 kb in length. Initial attempts to transmit the disease and/or the putative viral pathogen by mechanical inoculation have been unsuccessful, and nucleotide and amino acid sequence searches have found no significant identity to any known viral sequences.

Identification of a previously undescribed flexivirus causing island chlorosis of hackberry in Minnesota
B. E. LOCKHART (1)
(1) University of Minnesota
Phytopathology 98:S94

A virus with flexuous filamentous particles 760-800 nm in length was isolated from hackberry in Minnesota (*Celtis occidentalis*) showing symptoms of angular yellow leafspots similar to those described elsewhere for the disease known as island chlorosis of hackberry. The virus was transmitted readily by mechanical inoculation from infected plants to healthy hackberry seedlings which developed typical disease symptoms. A 1.7 kb fragment of the 3′ end of the viral genome was cloned and sequenced. The most significant amino acid sequence identity detected (34%) was to the polyprotein of *Grapevine leafroll-associated virus 3*.

Genomics of secondary metabolite production by *Pseudomonas fluorescens* Pf-5
J. E. LOPER (3), I. Paulsen (1), D. Bruck (3), M. Pechy-Tarr (5), M. Maurhofer (2), C. Keel (5), H. Gross (4)
(1) Macquarie University, Sydney, Australia; (2) Swiss Federal Institute of Technology, Zurich, Switzerland; (3) USDA-ARS, Corvallis, OR, USA; (4) University of Bonn, Bonn, Germany; (5) University of Lausanne, Lausanne, Switzerland
Phytopathology 98:S94

The soil bacterium *Pseudomonas fluorescens* Pf-5 is known to produce six secondary metabolites, and the genomic sequence of Pf-5 revealed three additional gene clusters, which encode for the biosynthesis of unknown natural products but contain conserved sequences of genes encoding for non-ribosomal peptide synthetases or polyketide synthases. Natural products synthesized from two of these orphan gene clusters have since been identified. Orfamide A, a novel cyclic lipopeptide produced by Pf-5, contributes to swarming motility of Pf-5 and has the capacity to lyse zoospores produced by phytopathogenic *Phytophthora* spp. Several analogs of rhizoxin, a macrocyclic lactone with antifungal activity, are synthesized from a large biosynthetic gene cluster in the Pf-5 genome. Linked to the rhizoxin biosynthetic locus is a cluster of genes encoding for an insect toxin termed FitD, which is related to Mcf (for "makes caterpillars floppy") produced by *Photorhabdus luminescens*, an inhabitant of the gut of entomopathogenic nematodes in the genus *Heterorhabditis*. If injected into the hemocoel, Pf-5 kills caterpillars of tobacco hornworms (*Manduca sexta*) whereas a *fitD* mutant of Pf-5 is less virulent. An oligonucleotide array representing each ORF in the Pf-5 genome has been developed, and is being utilized to explore the influence of the global regulatory genes *gacA*, *rpoS* and *ptsP* on transcription of secondary metabolite biosynthesis and efflux genes. The genomic sequence of *P. fluorescens* Pf-5 provides a variety of insights and into this organism's interactions with other organisms in the environment and biological control of plant disease.

Fungal endophytes from the Colombian Andean Paramo ecosystem inhibit the growth of plant pathogens *in vitro*
C. LOPERA (1), L. Avila (2), A. Rojas (2), A. E. Franco-Molano (1), M. C. Cepero (2), S. Restrepo (2)
(1) Universidad de Antioquia, Medellin, Colombia; (2) Universidad de los Andes, Bogota D.C., Colombia
Phytopathology 98:S94

Thirty fungal endophyte isolates were obtained from leaf segments of two plant species of the *Espeletia* complex (*Espeletia grandiflora* and *E. corymbosa*), which are endemic to the Cruz Verde Paramo, Colombia. We assessed the diversity of the isolates using morphological traits and sequencing of the ITS1-2 rDNA region. In order to detect those fungal endophytes able of producing secondary metabolites with antimicrobial activity, we analyzed the antagonistic *in vitro* activity of crude extracts against four plant pathogens (*Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Phytophthora infestans*). The crude extracts of some endophytes showed antimicrobial activity, reducing growth of plant pathogens *in vitro*. The antimicrobial effect of endophytic isolates inoculated on tomato plants grown *in vitro* was assessed by measuring disease severity. The presence and re-isolation of endophytes in plant allowed us to confirm the endophytic capacity of our isolates and their activity against the plant pathogens tested.

Characterization of the *rpoN* global regulatory gene of *Pseudomonas syringae* pv. *syringae* B728a and its impact on the plant-pathogen interaction
A. LORGE (1), D. Gross (1)
(1) Texas A&M University, College Station, TX, USA
Phytopathology 98:S94

Gene regulation in bacteria is highly complex and requires the activity of sigma factors that function as transcriptional regulators. In *Pseudomonas syringae* pv. *syringae* B728a, 14 sigma factors have been identified. One of the more interesting is rpoN, encoding sigma-54, which was initially described for its role in nitrogen utilization and later shown to be involved in regulating adhesion, motility, toxin production, and pathogenicity. The only commonality identified amongst these genes is that gene regulation by sigma-54 is not essential for normal growth and development because mutational inactivation of rpoN is not lethal. Unlike sigma-70, which recognizes promoter sites located at -10/-35 upstream of the transcription initiation site, sigma-54 recognizes sites located at positions -12/-24. *P. s. syringae* B728a encodes an RpoN that shares 80–98% identity with other *Pseudomonas* species. Promoter scans were conducted on the B728a genome to look for probable binding sites of RpoN. Analysis revealed that RpoN may be involved in regulating genes encoding ABC transporters, drug efflux pumps, flagella proteins, nitrate transporters, and several regulatory proteins. An insertional mutation in the rpoN gene was constructed in the B728a genome and a phenotypic analysis was initiated. Decreased swarming ability of the rpoN mutant was observed as compared to B728a. The ability to utilize sole nitrogen sources was also affected. The rpoN mutant showed little or no growth on sole nitrogen sources such as alanine, histidine, lysine, and serine. This study evaluated additional roles that RpoN plays in *P. syringae* B728a gene regulation and its involvement in the plant-pathogen interaction.

Comparison of the prevalence and incidence of *Soybean mosaic virus* in Iowa soybean fields during 2005 to 2007
X. LU (1), A. M. Robertson (1), E. Byamukama (1), F. W. Nutter Jr. (1)
(1) Iowa State University, Ames, IA
Phytopathology 98:S94

The wide-spread distribution of soybean aphid (*Aphis glycines*) in Iowa counties in recent years may result in an increasing risk for the prevalence and

incidence of viruses it can vector, including *Soybean mosaic virus* (SMV). A state-wide soybean disease survey was carried out in Iowa over the course of three growing seasons, beginning in May 2005, and concluding in September 2007. In each growing season, approximately 1,000 soybean fields were arbitrarily sampled at four growth stages: V2-V3, R1-R2, R4-R5, or R6-R7. Thirty soybean plants per field were collected using a systematic sampling design. The middle leaflet of the topmost fully developed leaf of each soybean plant was removed, and the 30 leaflets from each field were divided into five, 6-leaflet subsamples. Following sap extraction, samples were then tested for the presence of SMV by ELISA. By the end of 2007 growing season, 89 of the 99 counties sampled and tested were positive for SMV (89.9%), compared to 43 of 96 counties sampled in 2005 (32.3%) and 37 of 99 counties in 2006 (27.3%). The highest incidence of SMV in the positive counties was 46.0%, compared to 23.1% and 13.3% in 2005 and 2006, respectively. Based upon the survey data, there was weak or no association between the presence/ absence of SMV and the presence/absence of soybean aphid in the same field in the three growing seasons.

Quantification and comparison of components of aggressiveness of isolates of *Diaporthe phaseolorum* var. *caulivora* collected in Iowa soybean fields

X. LU (1), A. M. Robertson (1), F. W. Nutter Jr. (1)
(1) Iowa State University, Ames, IA
Phytopathology 98:S95

In recent years, an increase in the prevalence and incidence of northern stem canker in Iowa soybean fields has been reported by agronomists. One of the possible biological factors for this increase may be a change in aggressiveness of the causal agent, *Diaporthe phaseolorum* var. *caulivora* (DPC). As a part of the Iowa Soybean Disease Survey in 2005, 19 DPC isolates were isolated and single-spored from soybean plants exhibiting northern stem canker or pod and stem blight symptoms. To quantify and compare isolate aggressiveness, 9 of the isolates representing four different geographic areas in Iowa were arbitrarily selected for components analysis. Soybean cultivar "S35" was inoculated with each isolate at growth stage V2-V3 by inserting a single DPC-infested toothpick below the first trifoliate node. Each replication consisted of three 10-cm pots, each containing 4 plants. The entire experiment was conducted twice using the same controlled environmental conditions. Components of aggressiveness (incubation period, the rate of lesion expansion, final lesion length, and time to death) for each isolate and for isolates from the same geographic area were analyzed using analysis of variance (ANOVA), and mean separations were performed using the Waller-Duncan K-ratio test ($P \leq 0.05$). There were significant differences among the 9 isolates and among the isolates grouped from different areas for each of the components.

Strategies to reduce risk of benzimidazole resistance in *Monilinia fructicola* populations by using real-time PCR

Y. LUO (1), H. Reyes (1), D. Morgan (1), T. Michailides (1)
(1) Department of Plant Pathology, University of California-Davis, Kearney Agricultural Center, Parlier, CA, USA
Phytopathology 98:S95

Field inoculation experiments were conducted to study the effects of fungicide applications on changes in frequency of the E198A allele (FEA) that confers high benzimidazole-resistance in *Monilinia fructicola*, the causal pathogen of brown rot of stone fruit. Five treatments, no spray, 1, 2, 3, and 4 fungicide sprays during the season on immature peach fruit prior to inoculation were used. Three spore inoculum mixtures were used in the first inoculation: 90% benzimidazole-sensitive isolate S18 + 10% benzimidazole–resistant isolate R190, 50% S18 + 50% R190, and 10% S18 + 90% R190. Four sequential inoculations were made with inoculum from spores obtained from diseased fruit caused by the previous inoculation. A real-time PCR assay was applied to efficiently quantify the FEA for each combination of inoculum mixture and fungicide treatment and for each inoculation. The R190 was less competitive than S18 when no fungicide was applied, implying the instability of E198A allele in *M. fructicola* under natural conditions. However, increasing fungicide applications resulted in an increase in the FEA values at the end of the season. The quantitative changes in the FEA from various initial proportions of R190 under different fungicide treatments were analyzed. A table consisting of changes in the FEA values from early to late season resulted from the different fungicide applications was developed to provide strategies of benzimidazole resistance management.

A small family of *Phakopsora pachyrhizi* proteins localized to the cell wall

D. G. LUSTER (1), M. B. McMahon (1), M. L. Carter (1)
(1) USDA ARS FDWSRU
Phytopathology 98:S95

Phakopsora pachyrhizi, the causal agent of Asian soybean rust, has become established over an expanding range in the U.S. We previously identified and partially characterized a set of extracellular proteins recovered from *P. pachyrhizi* germination media. Two high titer proteins, PHEP107 and PHEP369 (for PHakopsora Extracellular Protein), were selected for further study. PHEP107 and PHEP369 are nearly identical in length (108 and 109 aa, respectively), and share 64% amino acid sequence identity (76% amino acid class similarity). Immunolocalization studies confirm an extracellular location for PHEP107 and PHEP369 in the spore and appressorial cell wall, but functional inhibition assays with antibodies have yet to identify a role for the proteins in fungal growth, development and/or infection. These proteins are not significantly similar to any sequences deposited in open source databases. PHAP369 was mapped to a set of fosmid clones generated from shotgun genomic sequencing of *P. pachyrhizi*. Analysis of *P. pachyrhizi* fosmid and EST sequences revealed four ORFs and one truncated EST with closely related sequence and intron/exon structure to PHEP 369, thus constituting a small protein family. No ORFs closely related to PHEP 107 were found in the fosmid library, but a PHEP 107 gene generated by PCR from *P. pachyrhizi* genomic DNA was 60% similar in sequence to PHEP369. Although both proteins contain distinct hydrophobic and charged domains near the N-termini, prediction algorithms do not reveal the presence of a canonical signal peptide for either protein. Yeast secretion trap assays with PHEP107 and PHEP369 were also negative. Results from Southern blot analysis will be presented to complete the analysis of gene structure and copy number of related members of the protein family.

Metabolic events that are important for soybean rust resistance

A. Lygin (1), S. LI (2), J. Widholm (1), V. Lozovaya (1)
(1) Department of Crop Sciences, University of Illinois at Urbana-Champaign, IL 61801; (2) USDA-ARS, Crop Genetics and Production Research Unit, Stoneville, MS 38776
Phytopathology 98:S95

Asian soybean rust, caused by *Phakospora pachyrizi* is an important new soybean disease that causes much concern at present in the U.S. agricultural community. There are no commercial soybean varieties with resistance to soybean rust at this time and U.S. cultivars are thought to be highly susceptible to this fungus. Efforts are underway to identify partial resistance or slow rusting traits. In order to understand the metabolic responses of soybean to *P. pachyrhizi* attack and to assist in development of cultivars with resistance to soybean rust, leaves of 14 days old soybean plants were inoculated with spore suspension of *Phakospora pachyrzi*. Leaf samples were taken 1 day, 1 week and 3 weeks after inoculation and soluble phenolic compounds were extracted by 80% methanol. Residues after extraction of soluble phenolics were used to prepare cell wall samples. Both soluble and cell wall-bound phenolics were analyzed by HPLC and spectrometry. We found that isoflavonoid and flavonoid synthesis in soybean leaves was strongly stimulated in response to rust infection. Several fold increases of flavonoids (quercetin and kempferol) and phenolic acid levels occurred in leaves of rust infected plants compared to the non-inoculated control. While the phytoalexin glyceollin and the precursor of glyceollin, daidzein were not detected in leaves of uninfected plants, both compounds accumulated at marked concentrations in rust infected leaves indicating that glyceollin is involved in the plant defense response to the soybean rust pathogen. Importantly, these compounds accumulated at much higher levels in plants that had better rust tolerance. The results obtained indicate that the soybean antibiotic glyceollin and cell wall-bound phenolics could be a good target for breeding and genetic engineering for resistance in soybeans to soybean rust disease.

A quantitative PCR assay for *Macrophomina phaseolina*

J. MA (2), J. S. Haudenshield (2), C. B. Hill (2), G. L. Hartman (1)
(1) USDA-ARS, Urbana, IL, USA; (2) University of Illinois, Urbana-Champaign, IL, USA
Phytopathology 98:S95

Charcoal rot of soybean is caused by *Macrophomina phaseolina* and crop damage may be considerable under conditions of high temperature or drought. This soilborne fungus has a wide host range, with numerous isolates infecting about 500 plant species in more than 100 families throughout the world. In order to develop a rapid and accurate DNA-based method for the specific detection of M. *phaseolina*, we selected the rRNA internal transcribed spacer (ITS) region as a target site, amplified this region from isolate MP3-3-1-1 using primers M1 and M4, and sequenced the resulting amplicon. That sequence was then aligned with the corresponding data available for other pathogenic fungi of soybean, and the primers Macr1 and Macr2 were chosen and synthesized, along with a dual-labeled linear hydrolysis probe MacrP1, to provide a 5'-fluorogenic exonuclease assay specific to *M. phaseolina*, isolate MP3-3-1-1. The DNA from nine other plant pathogenic fungi were tested and none produced a positive result, supporting the specificity of this assay. An endpoint dilution series of purified *M. phaseolina* total DNA exhibited accurate quantification using this technique to a detection threshold of

approximately 100 fg DNA. These results demonstrate that the assay we have developed can be a useful tool in rapid detection and quantification of *M. phaseolina*.

Fusarium comparative genomics
L. MA (1)
(1) The Broad Institute of Harvard and MIT
Phytopathology 98:S96

Collectively, Fusaria are the most important plant pathogens, causing disease in nearly every agriculturally important plant. To employ the power of comparative genomics in understanding pathogenesis of this group of organisms, three closely related species *F. graminearum*, *F. verticillioides* and *F. oxysporum*, have been sequenced and analyzed. The sequence conservation among these genomes allows an unprecedented ability to determine orthologs and to identify species-specific features. Over 90% of the *F. verticillioides* genome can be unambiguously aligned to the syntenic regions in *F. oxysporum* with an average 90% sequence identity. Specifically, all the eleven chromosomes in *F. verticillioides* have corresponding chromosomes in *F. oxysporum* and *F. graminearum.* In contrast, four of the *F. oxysporum* chromosomes, accounting for over 15 Mb, lack significant orthologous sequence in the other two genomes. These *F. oxysporum*-specific chromosomes are enriched for genes that encode secreted proteins including the known virulence factors such as, SIX (Secreted in Xylem) proteins and plant cell wall degrading enzymes. Transposable elements are also over-represented in these chromosomes. Examining sequence content and evolutionary mechanisms underlying the acquisition and diversification of such genetic material will open the door to understand the development of pathogenesis and host specificity.

***Colletotrichum fragariae* is a pathogen on hosts other than strawberry**
S. J. MacKenzie (1), J. C. MERTELY (1), T. E. Seijo (1), N. A. Peres (1)
(1) University of Florida, Wimauma, FL, USA
Phytopathology 98:S96

Evidence that *Colletotrichum fragariae* causes disease on hosts other than strawberry is limited. In the fall of 2006, fungal isolates from leaf spots on silver date palm and from leaf spots and stem lesions on cyclamen were tentatively identified as *C. fragariae*. After confirming pathogenicity of these isolates to their original hosts, a representative isolate from each host was compared to *C. fragariae* and to *C. gloeosporioides/Glomerella cingulata* isolates from strawberry. The cyclamen and date palm isolates bore tapered conidia in acervuli that were in the range of sizes described for *C. fragariae* reference isolates, and also produced conidia on setae. Sequence data from the combined internal transcribed spacer (ITS) regions 1 and 2 and the gene for the 5.8 ribosomal RNA from the cyclamen and date palm isolates matched the sequence for *C. fragariae* reference isolates. Based on these characters, it was concluded that the C. *fragariae* species designation was correct for both isolates. The cyclamen isolate and the strawberry reference isolates were similar in pathogenicity to strawberry crowns and the AT-rich DNA banding pattern of the cyclamen isolate was identical to the *C. fragariae* isolates from strawberry. However, the date palm isolate was less pathogenic to strawberry crowns and had a distinct AT-rich DNA banding pattern compared to all other isolates. These results indicate that: (1) *C. fragariae* is a pathogen on hosts other than strawberry and (2) diversity among *C. fragariae* isolates is greater than previously reported.

Evaluation of disease models for timing fungicide applications for control of anthracnose fruit rot of strawberry
S. J. MacKenzie (1), C. Moyer (1), J. Mangandi (1), T. Hasing (1), N. Proano (1), N. A. PERES (1)
(1) University of Florida, Wimauma, FL, USA
Phytopathology 98:S96

Anthracnose fruit rot, caused by *Colletotrichum acutatum*, is one of the most important strawberry diseases in central Florida and worldwide. Fungicide treatment regimes based on a disease model using the variables leaf wetness duration, temperature, and the presence or absence of disease symptoms were compared to a standard fungicide program for two seasons in Florida. High and low thresholds were selected to time applications of a preventive fungicide (captan) or a systemic fungicide (pyraclostrobin). Treatments were applied to Camarosa, a highly susceptible cultivar, and to Strawberry Festival, a less susceptible cultivar. Disease incidence in nontreated plots was higher for the 2007–08 season (~50% for Camarosa) than for the 2006–07 season (~15%). All the model-based treatments received fewer fungicide applications than the standard program. In general, applying fungicides at lower thresholds and prior to the occurrence of symptoms provided better control than applying fungicides at a higher threshold or after symptoms were present. Some model based programs provided comparable control to the standard fungicide program. The results will be used to develop an advisory system to help growers reduce sprays and production costs.

The distribution and epidemiology of *Phragmidium violaceum* (blackberry rust) in the western United States
W. MAHAFFEE (2), K. Johnson (1), T. Neil (2), C. Finn (2)
(1) Dept. Botany & Plant Pathology, Oregon State University; (2) USDA-ARS Hort. Crops Research Lab
Phytopathology 98:S96

In 2005, *Phragmidium violaceum* was first found on the Oregon coast. It caused extensive crop loss in *Rubus laciniatus* derived blackberry cultivars in Oregon and Washington. Surveys in 2005–2006 indicated that *P. violaceum* could be found on Rubus spp. below 640 m in elevation and west of the Sierra Nevada and Cascade mountains from Santa Cruz, CA to the Canadian border. All five spore stages were observed on commercial and feral blackberries over the course of the growing season. Disease severity was greatest in coastal areas were extended periods of fog are common. Disease development in the field mostly occurred during the spring rains with few new infections occurring until rain occurs in late August-September. The only commercial blackberries infected in the field were 'Thornless Evergreen' and 'Everthronless', with fields managed for annual harvest having more disease than those managed for alternate year harvest. *R. insularis* genotypes and crosses were also highly susceptible. Growth chamber experiments indicated that infection was limited when constant temperature was > 25°C with < 6 continuous hours of leaf wetness. When temperature regimes observed in the field were used, infection was limited by daily average temperature >20°C and < 6 continuous hours of leaf wetness. This information combined with climate data from the last 30 years indicate that severe blackberry rust epidemics will be sporadic and of minor importance in most years.

Differential and multiple host susceptibility (S) genes control the interaction of *Xanthomonas oryzae* pv. *oryzae* with the host plant rice (*Oryza sativa* L.)
T. MAHMOOD (2), G. Antony (2), B. Yang (1), F. White (2)
(1) Genetics, Development and Cell Biology, Iowa State University, Ames, IA; (2) Plant Pathology, Kansas State University, Manhattan, KS
Phytopathology 98:S96

Xanthomonas oryzae pv. *oryzae* delivers members of type III transcription activator-like (TAL) effectors into rice cells, where the proteins mediate gene/effector specific host gene expression. The pathogen is highly dependent on major TAL effectors, which mediated their effect through alternate targeted host susceptibility (S) genes. *X. oryzae* pv. *oryzae* contains 19 genes for TAL effectors and uses additional TAL effectors concomitantly as major, moderate or minor virulence effectors. These additional effectors target additional and specific host genes including *OsTFX1*, which encodes a bZIP transcription factor and *TFIIAgamma1*, which encodes the core transcription factor TFIIA gamma, or small, subunit. The roles of the plant genes in the host susceptibility are under investigation. The results indicate that *X. oryzae* pv. *oryzae* utilizes different yet related TAL effectors for multiple and concerted effects on host gene expression, and, as a consequence, host physiological responses as a strategy for virulence. Evidence regarding the different specificities of the TAL effectors in individual strains of the pathogen using immunological detection will be presented and discussed.

Mixed infection of *Alfalfa mosaic virus* and *Soybean mosaic virus* in soybeans results in disease synergism
M. MALAPI-NELSON (1), B. Ownley (1), K. Gwinn (1), M. Hajimorad (1)
(1) Dept. of Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN
Phytopathology 98:S96

Co-infection of potyviruses with taxonomically diverse plant viruses results in disease synergism and elevation in the level of the non-potyviruses involved. In the majority of cases, however, the level of the potyvirus remains essentially unchanged. A few potyviruses naturally infect soybean and it has been shown that *Soybean mosaic virus* (SMV) induces disease synergism in mixed infection with *Bean pod mottle virus* (BPMV). Soybean is naturally infected by a number of other non-potyviruses as well including *Alfalfa mosaic virus* (AMV) where its incidence in soybean growing areas of the Northern United States has been increased recently. AMV infection in soybean is associated with mild symptoms and symptom remission. We have now studied the interaction of SMV strains G2 (isolate N) and G7 with AMV strains JD and Ch in soybean cultivar Williams 82 and monitored the level of viruses in the infected tissues immunologically at 21 and 30 days post-inoculations. Mixed infection of soybean plants with both AMV and SMV was easily established by mechanical inoculation irrespective of simultaneous or sequential inoculation with the two viruses. In multiple mixed infection experiments the infected plants consistently exhibited disease synergism where the induced symptoms were distinct from infection by either virus alone in a strain-independent manner. Generally, the level of AMV was enhanced in mixed infection compared to the singly infected soybean plants,

which suggests AMV interaction with SMV is synergistic. Conversely, the level of SMV in the tissues exhibiting synergistic phenotypes was reduced compared to those infected with SMV alone suggesting antagonistic interactions between SMV and AMV. Nevertheless, the observations that co-infection of AMV and SMV results in induction of disease synergism suggests enhancement of the potential that AMV may become a serious viral disease of soybean.

Monitoring atmospheric transport of soybean rust spores into Minnesota

D. Malvick (1), C. FLOYD (1), S. Krupa (1), J. Kurle (1)
(1) University of Minnesota, Department of Plant Pathology, St. Paul, MN, USA
Phytopathology 98:S97

Soybean rust (SR), a destructive foliar disease of soybean caused by *Phakopsora pachyrhizi*, has been detected in the southern and central U.S. but not in Minnesota (MN). Before SR can develop in MN, urediniospores must be transported from source areas each year. To detect potential spore transport into MN and to validate components of a SR forecast system, passive, bulk spore deposition (both wet and dry) traps were located in 25 MN counties in 2007 and 24 counties in 2006. The spore traps, consisting of a 43 cm diameter open funnel with a micropore filter assembly attached at the base, were placed in soybean plots monitored weekly for SR infection. DNA was extracted from filters removed weekly from each spore trap and analyzed with a semi-specific, nested TaqMan quantitative PCR (qPCR) assay to detect the presence of SR spores (Barnes and Szabo, unpublished). Samples testing positive with the qPCR assay were analyzed further with DNA sequencing to confirm the presence of *P. pachyrhizi.* In 2006, SR spores were detected in 4 samples collected between July 31 and August 29 in Blue Earth (3 samples) and McLeod (1) counties. In 2007, SR spores were detected in 7 samples collected between June 22 and August 23 from Dakota (1), Freeborn (1), Jackson (1), Polk (2), Redwood (1), and Swift (1) counties. Pustules of SR were not detected on leaves analyzed microscopically from sentinel plots during either year. Results indicate that SR spores were transported to and deposited in MN during the growing season in 2006 and 2007, but that biological or environmental factors inhibited development of SR.

Monitoring management of huanglongbing disease of citrus in Brazil

K. L. MANJUNATH (4), R. Harakava (3), C. Ramadugu (5), P. Yamamoto (2), S. Halbert (1), R. F. Lee (4)
(1) DPI, Gainesville, FL; (2) Fundecitrus, Sao Paulo, Brazil; (3) Instituto Biologico, Sao Paulo, Brazil; (4) USDA-ARS, Riverside, CA, USA; (5) University of California, Riverside, CA, USA
Phytopathology 98:S97

Huanglongbing (HLB), or citrus greening disease, was first reported in Brazil in 2005. The disease is spread in nature by psyllid vector, *Diaphoriona citri*. *Candidatus* Liberibacter asiaticus and *Ca*. L. americanus have been found to be associated with the disease in Brazil. It is difficult to detect the presence of Liberibacters in plants early enough for successful management of the disease. A prolonged incubation period of two years or longer in some trees was indicated from a field trial. Selected blocks of citrus trees from three orchards practicing varying levels of disease management practices were selected for the present study. Weekly samples of psyllids were collected from these blocks over a period of one year and analyzed for the presence of both species of Liberibacters by real time PCR analysis. Selected samples were also analyzed by conventional PCR. Development of disease symptoms in these blocks was also monitored. Results show that the presence of Liberibacters can be identified in psyllids long before symptoms become visible in plants and show the usefulness of psyllid analysis in monitoring different management practices.

Resource development for efficient mapping of disease resistance traits in *Solanum*

H. S. MANN (1), E. A. Quirin (1), L. Gao (1), R. Aversano (3), M. Iorizzo (2), D. Carputo (3), J. M. Bradeen (1)
(1) University of Minnesota, Department of Plant Pathology, St. Paul, MN, USA; (2) University of Minnesota, St. Paul, MN, USA and University of Naples "Federico II", Portici, Italy; (3) University of Naples "Federico II", Portici, Italy
Phytopathology 98:S97

Species of the wild potato taxon superseries *Stellata* are potentially a rich source of genetic resistance to various potato pathogens. However, access to resistance (R) genes in these species has been limited by sexual incompatibility with cultivated potato and a lack of information on genomic structure. Towards improving access to R genes in *Stellata* species via gene cloning and transformation, our laboratory is characterizing genome structure in key species in this group. Currently, one of these species, *Solanum bulbocastanum* is being genetically mapped in our lab using Diversity Arrays Technology (DArT). Here we detail efforts to augment the burgeoning *S. bulbocastanum* genetic map with BAC clones harboring resistance gene homologs (RGAs). Several R genes have been cloned from the genus *Solanum* and the majority belong to the nucleotide binding site-leucine rich repeat (NBS-LRR) class. Conserved regions of the NBS domain of known R genes were used to develop primers to amplify RGAs from *S. bulbocastanum.* 107 RGAs thus identified were assembled into nine contigs at 90% sequence identity. PCR amplified DNA from the members of each contig was pooled and used as a probe to identify BAC clones harboring RGA sequences. DArT markers and PCR-based markers developed from BAC ends will be used to integrate the BAC clones into the *S. bulbocastanum* genetic map. The resulting integrated genetic and RGA physical map can be further used 1) for positional cloning of resistance genes, 2) as a resource for mapping additional resistance loci in a population-independent manner, and 3) as a resource for mapping disease resistance loci in other members of the of the superseries *Stellata* by exploiting their syntenic relationship with *S. bulbocastanum.*

Ptr ToxA alters photosystem I and II homeostasis prior to accumulation of reactive oxygen species

V. A. MANNING (1), A. L. Chu (1), J. E. Steeves (1), T. J. Wolpert (1), L. M. Ciuffetti (1)
(1) Oregon State University, Corvallis, OR, USA
Phytopathology 98:S97

Pyrenophora tritici-repentis, the causal agent of tan spot of wheat, produces host-selective toxins that act as pathogenicity/virulence factors. One of these toxins, Ptr ToxA (ToxA), is a protein toxin that induces cell death in ToxA-sensitive cultivars. The mechanism of cell death induction is beginning to be elucidated. It is known that ToxA enters the mesophyll cells of sensitive wheat cultivars and localizes to chloroplasts. In this study, we show that ToxA treatment of sensitive wheat cultivars results in reactive oxygen species (ROS) accumulation that occurs concomitantly with necrosis. ToxA-treated leaves held in constant dark, or treated with the anti-oxidant N-acetyl cysteine neither accumulate ROS nor display necrosis. Staining of whole leaves for ROS production followed by microscopy show that ROS production is occurring in the chloroplasts. Examination of complexes involved in photosynthesis via Blue native-gel electrophoresis followed by SDS-PAGE indicates that ToxA disrupts both photosystem I and II homeostasis irrespective of light conditions. Thus, current results suggest that ToxA selectively enters sensitive cells, localizes to chloroplasts, and disrupts photosystem function, leading to light-dependent ROS production and cell death. This project is supported by a grant from the NRI of the USDA CSREES.

Biological control of plant pathogenic fungi using *Talaromyces flavus*, *Sordaria fimicola* and some endophytic fungi

L. MANOCH (1), O. Jeamjitt (1), T. Dethoup (1), J. Kokaew (1), A. Eamvijarn (1), N. Visarathanonth (1), C. Chamswarng (2)
(1) Kasetsart University, Department of Plant Pathology, Faculty of Agriculture, Bangkok 10900, Thailand; (2) Kasetsart University, Department of Plant Pathology, Faculty of Agriculture, Kamphaeng Saen, Nakhon Pathom 73140, Thailand
Phytopathology 98:S97

Twenty isolates of *Talaromyces flavus* from soil were selected for antagonistic tests against 15 species of plant pathogenic fungi *in vitro* and in the greenhouse. All of the selected isolates of *T. flavus* inhibited the mycelial growth of *Phytophthora palmivora*, *P. parasitica*, *Peronophythora litchii*, *Colletotrichum capsici*, *C. gloeosporioides*, *Pestalotiopsis guepinii*, *Phyllosticta* sp., *Curvularia lunata*, *Helminthosporium maydis*, *H. oryzae* and *Fusarium oxysporum*However, none of the isolates could control . *Pythium aphanidermatum*, *Lasiodiplodia theobromae*, *Rhizoctonia solani* and *Sclerotium rolfsii in vitro*. However, in the greenhouse experiment 20 isolates of T. flavus controlled stem rot of mungbean, caused by *S. rolfsii*, 7 and 14 days after planting, and 6 isolates gave control up to 30 days after planting. In the second investigation, six isolates of *Sordaria fimicola* from various dung samples were used for antagonistic tests against nine species of plant pathogenic fungi *in vitro*. All isolates of *S. fimicola* could inhibit >50% mycelial growth of *Pestalotiopsis guepinii*, *Colletotrichum capsici*, *Curvularia lunata*, *Alternaria alternata* and *Fusarium oxysporum*. Four isolates of *S. fimicola* could inhibit *Lasiodiplodia theobromae*, whereas two isolates could control *Pythium aphanidermatum*. This fungus failed to control two Basidiomycetes anamorphs, *Rhizoctonia solani* and *Sclerotium rolfsii in vitro*. In the third investigation, two isolates of endophytic fungi from *Typhonium trilobatum* (ED1) and *Aegle marmelos* (ED2) inhibited *Pythium ultimum*, *Phytophthora cinnamimi*, *Sclerotinia sclerotiorum*, *Botrytis cinnerea*, *Verticillium dahlae*, *Ceratocystis ulmi*, *Colletotrichum lagenarium*, *Cercospora* sp. and *Bacillus subtilis.* However, these fungi could not inhibit *Candida albicans* and pathogenic yeast.

Understanding the genetic diversity of *Phytopthora cinnamomi* Rands using a multi-locus sequence based approach

M. A. MANSFIELD (1), M. Coffey (2), M. Peiman (2), D. M. Geiser (1), S. Kang (1)
(1) The Pennsylvania State University, Dept. Plant Pathology, University Park, PA 16801; (2) University of California Riverside, Dept. Plant Pathology, Riverside, CA 92521
Phytopathology 98:S98

The oomycete *Phytopthora cinnamomi* is one of the most destructive pathogens of woody perennials, causing extensive damage to Australian forests as well as the avocado industry in the U.S. Using isozyme migration patterns, Oudemans and Coffey (1) found that isolates from diverse geographic locations separated into eight electrophoretic types, suggesting that *P. cinnamomi* may contain cryptic species. To address this hypothesis, isolates of each electrophoretic type and additional isolates representing maximum host and geographic diversity were chosen from the World Phytophthora Collection and other sources. Based on the *P. sojae* genome, a close relative of *P. cinnamomi*, primers were developed to amplify five nuclear genes. Approximately 5.5 kb of sequence was generated for each isolate and 56% of this data consisted of intergenic sequence. Preliminary data suggest that despite geographic and host variation, *P. cinnamomi* is genetically uniform, with the major clade consisting of both A1 and A2 mating types and all eight electrophoretic types. However, we did discover a small subset of isolates that were distinct from the *P. cinnamomi* isolates as well as each other, suggesting that these may be novel species. Work is underway to evaluate these isolates. It is hoped that this information will be useful for resistance breeding programs as well as other management programs to control this difficult and widespread pathogen. (1) Oudemans, P. and Coffey, M.D.1991. Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. Mycol. Res. 95:19-30.

Discovery of new sources of resistance to *Moniliophthora perniciosa*, the witches' broom pathogen of *Theobroma cacao* in near isogenic lines of tomato

J. MARELLI (5), B. Masseret (4), V. Petiard (3), S. Tanksley (1), S. Grandillo (2), S. Maximova (5), S. Kang (5), M. Guiltinan (5)
(1) Cornell University, Ithaca, NY, USA; (2) Institute of Plant Genetics, Portici, Italy; (3) Nature Source Genetics, Ithaca, NY, USA; (4) Nestle R&D Centre, Tours, France; (5) The Pennsylvania State University, University Park, PA, USA
Phytopathology 98:S98

Theobroma cacao, a plant cultivated between the tropics, produces cocoa beans sold in the international market for the confection of chocolate. Witches' broom disease, caused by *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora, comb. nov.(=*Crinipellis perniciosa*) is among the diseases of foremost importance for the cacao industry because of its economical importance in South America. As an alternative host to cacao, tomato (*Solanum lycopersicum*) was used in inoculation experiments and showed susceptibility to the strains of the S-biotype (strain 73-6-01) of *M. perniciosa*. Comparatively, the tomato wild relative, *Solanum habrochaites*, was highly resistant to the disease when exposed to the same strain of *M. perniciosa*. One hundred near isogenic lines of tomato containing chromosomal sections of the genome of *S. habrochaites* in a *S. lycopersicon* background were used to identify genome regions of *S. habrochaites* that could be responsible for the observed resistance to *M. perniciosa*. A strong QTL for resistance on the short arm of chromosome 1 was detected for disease severity ($P < 0.05$), shoot diameter ($P < 0.001$), and shoot fresh and dry weight ($P < 0.0001$). That genomic area is known to contain a cluster of genes for resistance against the pathogen *Cladosporium fulvum*. Implications for improving cacao breeding strategies will be discussed.

Molecular identity, infectivity and differential gene expression associated with an *Olpidium*-like fungus in citrus and vegetables

S. Marepally (1), M. Kunta (1), J. V. Da Graca (1), S. Nelson (2), M. SKARIA (1)
(1) Texas A&M University-Kingsville Citrus Center, Weslaco, TX, USA; (2) Texas A&M University-Kingsville, Kingsville, TX, USA
Phytopathology 98:S98

Citrus psorosis disease is widespread and a limiting factor in citrus production, worldwide. It is suspected to be transmitted by a natural vector besides grafting, the major mode of disease transmission. *Olpidium*-like fungus was isolated from roots of psorosis infected trees in an orchard in the south research farm of Texas A & M University-Kingsville Citrus Center. Tomato, cabbage and lettuce seedlings were inoculated with zoospores for infectivity studies. Genomic DNA was isolated from zoospores and molecular detection of zoospores was carried out by polymerase chain reaction (PCR). Three fragments of sizes approximately 300 bp, 750 bp and 1100 bp were amplified using the primers NS7-NS8, ITS4-ITS5 and NS7-ITS4, respectively. These fragments will be cloned and sequenced. *Olpidium*-like zoospores were inoculated into 6 month-old seedlings of sour orange and C-22 (a trifoliate hybrid) rootstocks. Zoospores started settling onto the seedlings within 2 h of inoculation. Hourly observations for 8 h showed that the number of zoospores and resting spores established on C-22 seedlings were more than in sour orange rootstock. Total RNA was extracted from root sample after 2 h and 8 h of inoculation and double stranded complementary DNA (cDNA) was synthesized. The differentially expressed genes are being analyzed by amplified fragment length polymorphism (AFLP). Primers, E-ACC and M-CAA produced a successful DNA finger printing profile.

Biological differences among *Pectobacterium* species

M. MARQUEZ-VILLAVICENCIO (1), J. Glasner (2), N. Perna (2), A. Charkowski (1)
(1) Department of Plant Pathology, University of Wisconsin - Madison, WI, USA; (2) Genome Center of Wisconsin, University of Wisconsin - Madison, WI, USA
Phytopathology 98:S98

Previously we described the genome sequences of two *Pectobacterium* spp. belonging to distinct geographical regions and phylogenetic clades. Genome differences were identified between *P. carotovorum* WPP14 (*Pc*) and *P. brasiliensis* 1692 (*Pb*) which may explain their divergent evolution and variation in life style, pathogenicity and survival. We now have extensive genetic information for multiple species of soft rot pathogens; however limited data is available describing the biology of these pathogens and environmental effects on disease development. Our research objective was to investigate the relative aggressiveness of *Pectobacterium* spp. in potato tubers and relative adherence ability to plant tissue. Although regulatory networks and cell wall degrading enzymes are homologous among species, we found in tubers inoculated with 10^6 CFU/ml and incubated at 20°C and 28°C, *P. atrosepticum* (*Pa*) was significantly less virulent than *Pc*. An additional 3 strains clustered with each species were also tested to confirm this result was not strain specific. On potato tubers inoculated with as little as 10^5 CFU/ml only *Pb* was able to cause lesions on potato. Interestingly, these species also differed in their ability to adhere to alfalfa sprouts as *Pc* showed a 10-fold increase in attached bacteria compared with *Pb* and 100-fold when compared with *Pa*. With the genome sequence available the future challenge is to determine the molecular basis of these phenotypes, linking them to alternative genes or regulation pathways currently unknown.

Relationships of bacterial strains causing heart rot of pineapple to *Dickeya* species based on 16S-23S intergenic spacer and *dnaA* sequences

G. Marrero (1), K. Schneider (1), A. ALVAREZ (2)
(1) University of Hawaii Manoa, Dept. of Molecular Biosciences and Bioengineering; (2) University of Hawaii Manoa, Dept. of Plant and Environmental Protection Sciences
Phytopathology 98:S98

Bacterial heart rot of pineapple is a serious disease worldwide and an accurate identification method for the pathogen is needed for quarantine purposes. In Hawaii, the disease was first reported in December, 2003, when it was attributed to *Erwinia chrysanthemi*. Subsequent taxonomic revision of the genus has placed *E. chrysanthemi* into the genus *Dickeya*, which comprises six species. Molecular markers were assessed for their ability to discern the relationship of the Hawaiian pineapple strains to known *Dickeya* species. The intergenic spacer (ITS) region between the 16S and 23S rRNA genes is known to be highly variable in length and sequence and has been used to discriminate closely related bacteria. The ITS region of 43 strains, including isolates from pineapple, irrigation water, other plant hosts and *Dickeya* type strains were sequenced and compared. Sequence information from the bacterial chromosomal replication initiator gene, *dnaA*, were also compared in this study. Phylogenetic trees, constructed using the neighbor-joining method, showed low bootstrap support for the relationships of the strains when ITS sequence data was used. However, the opposite was true for *dnaA*. High bootstrap support of 98 and 91 percent using neighbor-joining and maximum parsimony methods, respectively, were observed when trees were constructed using *dnaA*. Preliminary data based on the *dnaA* molecular marker indicate that pineapple strains are more closely related to *D. dadantii*. This marker shows significant promise for use as a species level identifier and may serve as a confirmatory test in issues related to disease outbreaks, quarantine and regulatory processes.

Protein interaction and localization maps for plant-adapted rhabdoviruses

K. MARTIN (2), A. Bandyopadhyay (2), J. Wigginton (2), R. Dietzgen (1), M. Goodin (2)
(1) Department of Primary Industries and Fisheries 80 Ann St, Brisbane, Queensland, Australia; (2) University of Kentucky, Lexington, KY, USA
Phytopathology 98:S98

Sonchus yellow net virus (SYNV) and *Potato yellow dwarf virus* (PYDV) are important in studies to elucidate the mechanism(s) by which members of the

genus Nucleorhabdovirus elicit dramatically different effects in a common host, *Nicotiana benthamiana*. Prior to the construction of interactome maps composed of host factors and viral proteins, we have determined the in planta associations for the nucleoprotein (N), phosphoprotein (P), putative movement protein (sc4), matrix protein (M) and glycoprotein (G) of SYNV in all pairwise combinations. Protein interactions were assayed by bimolecular fluorescence complementation using novel binary vectors for facile recombination-based cloning. To complement the interaction data, we have mapped the functional domains in the SYNV-G protein using localization of green fluorescent protein fusions. Our deletion analyses show that targeting of SYNV-G to ER/perinuclear membranes requires the transmembrane domain but not the carboxy-terminus. However, the carboxy-terminus of SYNV-G contains at least one functional nuclear localization signal (NLS) that is capable of directing GFP to the nucleus. These NLSs may be required for targeting G protein to the inner nuclear membrane, a requirement for viral morphogenesis. Finally, we extend previous interaction data for the SYNV-N and -P proteins by demonstrating that these proteins are at least dimers *in vivo*. Coexpression of the N and P proteins of both SYNV and PYDV show similar associations in sub-nuclear loci. However, in contrast to the SYNV proteins, the PYDV-N and -P proteins lack predictable NLSs, suggesting that different mechanisms for nuclear import are employed by these viruses. Comparative analyses with the Cytorhabdovirus *Lettuce necrotic yellows virus* are under way.

Strawberry virus survey in the United States and Canada

R. R. MARTIN (2), I. E. Tzanetakis (1)
(1) Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR, USA; (2) USDA-ARS Horticultural Crops Research Lab, Corvallis, OR, USA
Phytopathology 98:S99

In an effort to determine the distribution of strawberry viruses in the U.S. and Canada, approximately 1500 samples were collected and either brought back or shipped to the USDA-ARS laboratory in Corvallis between 2002 and 2007. RNA was extracted from leaf tissue and archived at –80C for subsequent uses. During the same time, RT-PCR tests were developed for most known strawberry viruses. For this study 275 samples representing the major strawberry production areas in the U.S. and Canada were tested for: Beet pseudo yellows (BPYV), Fragaria chiloensis latent (FClLV), Strawberry crinkle (SCV), Strawberry latent ringspot (SLRSV), Strawberry mottle (SMoV), Strawberry mild yellow edge (SMYEV), Strawberry necrotic shock (SNSV), Strawberry pallidosis (SPaV) and Strawberry vein banding (SVBV) viruses, as well as a housekeeping gene as an internal control by RT-PCR. The Pacific Northwest had the highest rates of infection with the aphid-borne viruses but was virtually free of the whitefly transmitted viruses. In contrast, California, the southeastern U.S., the northeastern U.S. and Ontario, and the Midwest had aphid and whitefly transmitted viruses in about equal numbers, with the Midwest having the lowest incidence of virus infection. BPYV was only found in the samples from CA and the southeastern U.S. in these samples but has been detected from Maryland in previous studies. In the Pacific Northwest, fields with aphid control had very low incidence of virus infection compared to nearby fields without aphid control. Also the disease pressure was much lower in Oregon than in northern Washington or British Columbia.

Influence of temperature on the development of the heart rot disease of the agave *Agave tequilana* Weber Var. Azul

J. MARTINEZ-RAMIREZ (2), P. Posos-Ponce (2), F. Bernache-Pérez (1), S. Mena-Munguía (2), L. Rendón-Salcido (2), J. Vázquez-Navarro (2), J. Santillán-Santana (2)
(1) Estudiante graduado Departamento de Producción Agrícola, CUCBA, Universidad de Guadalajara, Zapopan, Jalisco, México; (2) Profesor Departamento de Producción Agrícola, CUCBA, Universidad de Guadalajara, Zapopan, Jalisco, México
Phytopathology 98:S99

The agave *Agave tequilana* Weber Var. Azul, raw material for the elaboration of the well-known "Tequila" has been strongly affected by diseases one of the most important is the heart rot, caused by a complex of bacteria and fungus. During the months of October to March of the years 1998–1999 and 2003–2004, in the municipality of El Arenal, Jalisco, Mexico, experiments were carried out to determine the influence of the temperature on the development of the disease. The plants studied were rated using a individual scale of damage proposed by Martínez-Ramírez et al 1997. The qualifications were repeated at intervals from 7 to 10 days during the study. The results indicated that temperatures below 10°C increased the disease significantly. Epidemics of 1998–1999 and 2003–2004 of agave populations were compared by using regression analysis, A polynomial mathematical model was obtained that is adjusted to both epidemics y = 0.0026X2 + 0.2949X + .9508, (y = percent of plants diseased, X = days to pass with temperatures below 10°C).

***Cucumber mosaic virus* (CMV)-induced symptoms in bell pepper (Capsicum annuum L.)**

J. MASIRI (1), R. R. Dute (1), J. F. Murphy (1)
(1) Auburn University, AL, USA
Phytopathology 98:S99

Cucumber mosaic virus (CMV)-induced symptom types are dependent on the host plant species and variety, virus strain, time of inoculation, and environmental conditions. In susceptible 'Early Calwonder' pepper plants, symptom development of CMV occurred in three phases associated with host plant development stages. The initial expression of systemic symptoms occurred in young leaves in the form of a chlorosis over the basal half of the leaf. As new leaves emerged and expanded, a second phase of symptoms developed consisting of a chlorotic mosaic over the entire leaf. Leaves expressing these symptoms had high titers of CMV, based on ELISA, with correspondingly high virus titers in the stem when tested by immuno-tissue blot analysis. It was during this mosaic symptom phase that stunting occurred which consisted of reduced extension of internodes associated with no significant effects on leaf numbers and aboveground fresh weight. Microscopic evaluation of symptomatic leaves revealed a changed cellular organization relative to comparable healthy control samples. As the primary stem initiated branching, at approximately the 10 to 12 leaf stage, newly emerged leaves were dull green in color, had varying degrees of deformation and were smaller than comparable healthy leaves. Subsequent leaves that emerged expressed only mild, if any, symptoms with stem elongation being comparable to healthy plants. CMV accumulation levels in these mildly symptomatic leaves and the stem were significantly less than observed during early phases of symptom development. Microscopic evaluations of leaves in the "recovered" part of the plant indicated a cellular content and organization similar to leaves from a healthy control.

Locating resistance QTL for Fusarium head blight using association mapping in contemporary barley breeding germplasm

J. M. MASSMAN (1), K. P. Smith (1)
(1) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S99

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is currently one of the most serious diseases of barley. Recent epidemics have led to intense breeding efforts to improve host resistance, progress however has been slow. Previously described resistance QTL (identified in wide bi-parental mapping studies) are often associated with negative agronomic traits such as late heading and taller plants, thus reducing their overall utility. Mapping within existing breeding populations, however, would identify those resistance QTL segregating in more adapted germplasm, and represent genetic factors that are immediately available to breeders. Association mapping has previously been effective at mapping within complex structured populations. Briefly, association mapping, leverages the numerous historical meiotic events in a crop's history to map QTL, with potentially better resolution than bi-parental mapping. The objective of this study was to utilize association mapping to identify markers associated with FHB resistance. To achieve this goal, 384 breeding lines from four barley breeding programs (UM, Busch Ag, and two from NDSU) were evaluated for resistance in five total environments over 2006/2007. At each location the lines were planted in a randomized complete block design with two replications, inoculated, and mist irrigated to encourage disease development. Each line was genotyped using 1536 single nucleotide polymorphism (SNP) markers, and QTL were mapped using a mixed model approach. Phenotypic variation among lines for disease severity was significant ($P < 0.0001$), but skewed toward resistant. Linkage disequilibrium extended more than 10cM on average, indicating this method was appropriate with available marker density. Population structure was accounted for using molecular marker and pedigree data. Multiple QTL with generally small effects ($R^2 < 8\%$) were identified. These loci should be useful targets for immediate implementation of marker assisted selection to improve disease resistance.

Effectiveness of the biopesticides Actinovate and Kaligreen within a management program for powdery mildew on cantaloupe

M. E. MATHERON (1), M. Porchas (1)
(1) The University of Arizona, Yuma, AZ, USA
Phytopathology 98:S99

Powdery mildew on cantaloupe and other melon crops, caused by the fungus *Podosphaera xanthii*, can result in significant yield losses. A field trial was conducted to compare the efficacy of a new biopesticide Actinovate (*Streptomyces lydicus*), an established biopesticide Kaligreen (potassium bicarbonate) and the conventional fungicide Procure (triflumizole), applied alone or within a rotation program with each other, for control of powdery mildew on cantaloupe. Most treatments were applied five times at weekly intervals to each of the five replicate plots arranged in a randomized complete

block design. Powdery mildew was not observed in plots until the third treatment application date; however, a high level of disease developed on nontreated cantaloupe plants by crop maturity. Disease ratings at plant maturity revealed that reduction of powdery mildew due to five applications of the biopesticides Actinovate or Kaligreen alone at weekly intervals was 72 and 59%, respectively, whereas similar application timing for the conventional fungicide Procure resulted in 100% disease control. In treatment programs where the conventional fungicide was alternated with one of the biopesticides, reduction of powdery mildew by Procure alternated with Actinovate or Kaligreen was 82 and 85%, respectively. Alternate application of Actinovate and Kaligreen reduced final disease severity by 79% compared to nontreated plots. These preliminary results suggest that a treatment program alternating application of the conventional fungicide Procure with either of the biopesticides Actinovate or Kaligreen could provide a high level of powdery mildew control on melon crops. Further evaluation of these products is in progress.

Detection and identification of Fusarium species in field pea roots

F. M. MATHEW (2), T. Barasubiye (1), S. G. Markell (2), R. S. Goswami (2)
(1) Agriculture and Agri-Food Canada, Ottawa, ON, K1A 0C6; (2) Dept. Plant Pathology, North Dakota State University, Fargo, ND 58105
Phytopathology 98:S100

With a significant expansion in acreage of field pea (*Pisum sativum*) in North Dakota, incidence of root rots has increased over the past few years. This led to a study on the identification of major root pathogens of field peas in North Dakota. Analysis of a collection of symptomatic root samples from five locations in the state conducted during the summer of 2007 resulted in the detection of several *Fusarium* spp., *Ascochyta pinodes* and *Rhizoctonia solani*. Diversity of Fusaria present on affected roots was studied using a DNA array based on sequences from the EF-1 region developed for identification of *Fusarium* spp. Eleven different species of Fusarium were detected using this technique. Among these, *Fusarium solani*, *F. oxysporum*, *F. avenaceum*, *F. redolens*, *F. graminearum*, and *F. acuminatum* were isolated from the affected roots and their identity confirmed by morphological characteristics and sequence comparisons. Pathogenicity tests to assess capability of these species to induce root rot are currently in progress. These findings suggest the possible involvement of a number of *Fusarium* species in the development of root rot, some of which have not been reported previously in field peas.

Global gene expression analysis of *Magnaporthe oryzae* under stress conditions

S. M. MATHIONI (2), C. Rizzo (1), N. M. Donofrio (2)
(1) Agilent Technologies; (2) University of Delaware
Phytopathology 98:S100

Rice blast disease, one of the most devastating diseases of rice, is caused by the ascomycete fungus *Magnaporthe oryzae*. During its infective life cycle, this fungus potentially encounters a variety of stresses such as oxidative environments, temperature shifts, and nutritional limitations. Analyzing the transcriptome of *M. oryzae* during such conditions, may reveal the molecular mechanisms employed by the pathogen to overcome potential plant-induced stresses, hence contribute to pathogenicity. We performed a global microarray gene expression analysis of *M. oryzae* under various *in vitro* stresses including oxidative induced by methyl viologen, heat-shock and nutritional limitation. These conditions were compared to the transcriptomes of *M. oryzae* growing in planta in both rice and barley detached leaves during invasive growth. All conditions were compared to the fungus grown under ideal growth conditions (WT). We found a total of 287 differentially expressed genes (17 up- and 270 down-regulated) common for all treatments in comparison to WT. Their putative functions are related to electron and heavy metal transport, lipid and sugar metabolism, oxidoreductase activity, signal transduction, and cell organization. Ongoing experiments of validation of several genes with putative functions in DNA binding, spermidine biosynthesis, transcriptional activation and virulence, by quantitative real-time RT-PCR have thus far confirmed the microarray data set. Future knockout experiments will further confirm the function of these and other genes, and their involvement in stress conditions.

Development and validation of a tissue based panel for the *P. ramorum* proficiency testing program

V. A. MAVRODIEVA (1), S. Negi (1), D. Picton (1), L. Levy (1), P. Tooley (2), N. Shishkoff (2), D. Luster (2)
(1) USDA APHIS PPQ CPHST, Beltsville, MD, USA; (2) USDA ARS FDWSRU, Fort Detrick, MD
Phytopathology 98:S100

Proficiency testing (PT) is a key element of laboratory accreditation program. A tissue-based PT panel for *P. ramorum*, used by the National Plant Protection Laboratory Accreditation Program (NPPLAP), was developed and validated in 2008 to assess proficiency of diagnosticians at critical stages of the diagnostic for *P. ramorum.* Healthy and *P. ramorum* infected *Rhododendron* 'Cunningham's White' leaves were used to prepare PT samples by lyophilization. Detached rhododendron leaves were dip-inoculated with 5000 sporangia per ml of *P. ramorum* and incubated in plastic moist chambers for 4–7 days at 20°C in darkness. Each batch of lyophilized tissue was characterized by DNA extraction and real-time PCR (TaqMan) analysis of 5–10% of the PT panel samples. Mean (average) Ct values and standard deviation coefficients (STDV) were estimated for the *P. ramorum* (FAM) and plant DNA (Texas Red) markers. To create PT tissue samples at varying concentrations, *P. ramorum*-infected tissue was diluted with healthy tissue at different ratios. The batch of 1:9 infected/healthy tissue ratio had a low STDV and produced a mean FAM Ct approximately 3.3 cycles higher then the undiluted *P. ramorum* infected batch. At greater ratios (1:99 and 1:999), STDVs were 3.42 and 3.96 respectively. These samples were not used for the panel. Alternatively, healthy plant tissue was spiked with *P. ramorum* culture DNA to produce low-level infection samples. Using this method we obtained a batch with a high mean FAM Ct and satisfactory STDV. Selected PT sample batches were then validated by three analysts to determine PT panel performance. PT panel stability was monitored monthly.

Susceptibility of peaches (cv. Chimarrita) at different ages to *Monilinia fructicola* infection

L. L. MAY-DE MIO (3), L. Amorim (1), F. Fayad (2)
(1) ESALQ/USP, Piracicaba, São Paulo, Brazil; (2) UFPR, Cutitiba, Paraná, Brazil; (3) Universidade Federal do Paraná, Curitiba, Paraná, Brazil
Phytopathology 98:S100

Brown rot (*Monilinia fructicola*) is the most important disease of peaches in Brazil. Its control demands a fungicide-spraying program during plant blooming and before harvest. However, this spraying schedule is not sufficient to control the disease in cv. Chimarrita. The objective of this research was to determine the susceptibility of fruits from different ages to the pathogen infection. The experiment was carried out in 24 four-year-old trees of cv. Chimarrita. Ten fruits in each plant were inoculated when they reached different diameters (i) 0.6-1.2 cm, (ii) 1.5-2.0 cm, (iii) 2.5-3.0 cm, (iv) 3.5-4.0 cm, (v) 4.0 – 5.0 cm, and (vi) >5.0 cm. Additionally, two control treatments were applied: non inoculated fruits exposed to the natural inoculum and non inoculated fruits protected from infection when they reached 0.6 cm diameter. The inoculation was done by the deposition of 10 ml of conidial suspension (10^6 conidia.ml^{-1}) on the surface of each fruit. Immediately after the inoculation the fruits were involved in a paper bag. After the harvesting, fruits were incubated under light at 25°C for 10 days. Disease incidence was assessed daily and plotted against time in order to draw disease progress curves. Disease incidence in control treatment was low (1% in bagged fruit and 2% in fruit exposed to natural inoculum). The shapes of disease progress curves were similar for all treatments, but the asymptote levels were different. Treatments (i) and (ii) showed the lowest disease incidence levels (2.5 and 3.6%), followed by treatments (iii) and (iv) (6 and 6.9%) and treatments (v) and (vi) (14.8 and 20%). Infection by *M. fructicola* in Chimarrita occurs in fruits from 0.6 cm diameter until ripening stage. The efficiency of infection increases with the age of the fruit.

Role of cyclic lipopeptide surfactants in bacterial defense against protozoan predation

M. MAZZOLA (1), J. M. Raaijmakers (2)
(1) USDA-ARS; (2) Wageningen University
Phytopathology 98:S100

Cyclic lipopeptides (CLPs) are produced by a diversity of bacterial genera, possess biosurfactant activity, and play a key role in a multiplicity of natural functions for the producing bacteria, including swarming motility and biofilm formation. CLPs have the capacity to disrupt membrane integrity leading to the cell lysis of certain microbial life stages, including oomycete zoospores. This study shows that the CLPs massetolide A and viscosin produced by the bacteria *Pseudomonas fluorecens* strains SS101 and SBW25 lead to lysis of protozoan trophozoites and confer protection from predation by the amoeba-flagellate *Naegleria americana*. *In vitro*, massetolide A-producing SS101 and viscosin-producing SBW25 were significantly less susceptible to grazing by N. americana than the corresponding CLP-deficient *massA* mutant 10.24 and *viscA* mutant of SBW25. Genetic complementation of the *massA* mutation in 10.24 restored massetolide A biosynthesis and protection from *N. americana* predation. Populations of mutant 10.24 exhibited a more rapid decline in bulk soil relative to the parental strain, with SS101 maintaining a population approximately one order of magnitude greater than the surfactant-deficient mutant two weeks post-inoculation. Collectively these results show, for the first time, that CLPs produced by *Pseudomonas* contribute to survival in soil and are potent metabolites in the bacterial defense against protozoan predation.

Understanding differential virulence within *Fusarium virguliforme* using multiloci fingerprint analyses

G. C. MBOFUNG (1), T. C. Harrington (1), J. Steimel (1), X. Yang (1), S. S. Navi (1), L. F. Leandro (1)
(1) Iowa State University
Phytopathology 98:S101

Sudden death syndrome, caused by *Fusarium virguliforme*, is presently the second most damaging disease affecting soybean production in the Midwest. Although isolates of the pathogen have consistently exhibited differing aggressiveness on susceptible soybean cultivars, DNA sequence analyses of multiple loci have demonstrated a genetically homogenous population in the USA. To further investigate the genetics behind this variation in virulence, fingerprint analyses were conducted on isolates originating from both Iowa and Minnesota. A total of 80 isolates were analyzed targeting four different loci. Inter-simple sequence repeat primers M13, T3B, $(GTG)_5$, and $(GACA)_5$ yielded identical fingerprint patterns for all isolates. Twelve RAPD primers resolved the isolates into seven genotypes. The use of $(CAT)_5$ and $(CAC)_5$ as fingerprint probes also delineated the isolates into five genotypes. About 80% of isolates were identical, while isolates belonging to the other four groups differed depending on the locus under consideration. The different groups did not correlate well to area of origin. Representatives of each genotype were tested for aggressiveness on a susceptible soybean variety. The correlation of aggressiveness on soybean with DNA polymorphisms based on the different loci will be discussed.

Quantification of *F. virguliforme* in field soil using TaqMan real-time polymerase chain reaction

G. Mbofung (1), A. FESSEHAIE (1), L. Leandro (1)
(1) Iowa State University
Phytopathology 98:S101

Fusarium virguliforme is the cause of soybean sudden death syndrome (SDS). The disease accounts for second highest yield losses in the Midwest of the United States. Classical dilution plating methods to determine inoculum density in native soil resulted in inconsistencies due to slow growth of the pathogen and variable colony morphology. The use of DNA quantification may be a more reliable method to assess pathogen presence and density in soil. The objective of this study was to use TaqMan PCR assay to quantify and determine the pattern of distribution of *F. virguliforme* in soil, and compare the pathogen levels with SDS intensity on soybean varieties. TaqMan PCR was used to quantify *F. virguliforme* DNA in soil samples collected at planting. Total soil DNA was extracted from soil samples using a soil kit. Real time PCR was performed using an existing TaqMan assay for *F. virguliforme*. Five independent DNA extractions were done per soil sample. Sterile and nonsterile soil was spiked with a ten-fold spore dilution and used as a standard for absolute quantification. *F. virguliforme* spore counts per gram of soil for each sample were determined by extrapolating from the calibration curve. The 18S rDNA TaqMan assay kit was included as an internal amplification control to account for PCR negative signals. *F. virguliforme* was detected in more than 83% of the soil samples. Results also showed that the pathogen was randomly distributed in the plots. Some plots with high spores density showed higher disease severity in the susceptible cultivar. In contrast, the resistant cultivar exhibited very low disease severity irrespective of the spore counts. We conclude that the TaqMan PCR method allowed quantification of the pathogen DNA in soil. This approach can be used to estimate SDS pathogen levels in a field prior to planting. PCR data can be used to develop SDS control and management strategies.

Characterization of *bruI*, an *expI*-like autoinducer synthase gene, in *Brenneria rubrifaciens*, the causal agent of deep bark canker on walnut

A. E. MCCLEAN (1), B. A. Duerkop (2), E. Greenberg (2), D. A. Kluepfel (1)
(1) USDA, ARS, Crops Pathology Genetics Research Unit, Davis, CA, USA; (2) University of Washington, Department of Microbiology, Seattle, WA, USA
Phytopathology 98:S101

Deep Bark Canker (DBC) is a disease that affects mature English walnut cultivars and is caused by the bacterium, *Brenneria rubrifaciens*. To identify genes involved in virulence and production of the pigment, rubrifacine, a pool of transposon mutants was generated and screened *in planta* and *in vitro*, respectively. Pigment minus mutants could be separated into three categories based on sequence homologies of host DNA adjacent to transposon insertion sites. The three classes were: 1) a non-ribosomal peptide synthetase (NRPS) like region, 2) a *hor*-like transcriptional regulator gene, and 3) a gene designated *bruI*, a *luxI* family acyl-homoserine lactone synthase gene homologous to *expI* from *Erwinia carotovora*. Characterization of the *bruI* minus mutant revealed a loss of HR elicitation in tobacco and attenuation of disease symptoms on English walnut. Using C18 reverse phase high performance liquid chromatography (HPLC) and a quantitative bioassay, the main *bruI* signal molecule was shown to be N-3-oxo-hexanoyl-homoserine lactone (3OC6-HSL). *In vitro*, two *Brenneria* species, *B. nigrifluens* and *B. salicis*, as well as synthetic 3OC6-HSL complemented the pigment minus phenotype of *B. rubrifaciens bruI* inactivated mutants. Discovery of how *B. rubrifaciens* regulates virulence via quorum sensing will enhance our ability to develop strategies that interrupt the long latency period of this bacterium.

Balancing nitrogen and fungicide applications to minimize carrot leaf blights

M. MCDONALD (1), S. M. Westerveld (1), C. Saude (1)
(1) University of Guelph, Guelph, ON, Canada
Phytopathology 98:S101

Alternaria leaf blight (A. dauci (Kühn) Groves and Skolko) and Cercospora leaf spot (C. carotae (Pass.) Solheim) are managed together with an extensive fungicide spray program, but there is the potential to reduce fungicide applications through nitrogen (N) application. Every combination of three rates of N (0, 110 and 220 kg/ha) and three fungicide treatments (0, 3 and 5 sprays) were applied to carrots grown on mineral soil in Ontario, Canada in 2006 and 2007. The severity of both diseases was assessed biweekly throughout the season and at harvest. Total and marketable yield were assessed at harvest. The area under the disease progress curve for both diseases decreased with increasing number of fungicide sprays in 2006, but was unaffected by N application. In 2007, the area under the disease progress curve for each disease was lowest when carrots received 220 kg/ha N and three or five fungicide sprays, and highest when carrots received no N and no fungicides. Carrots treated with the high rate of N and no fungicides had the same level of Alternaria leaf blight as carrots treated with no N and three or five fungicide sprays. Disease severity at harvest decreased with increasing number of fungicide sprays and decreased slightly with increasing N application in both years. Total and marketable yield increased with increasing number of fungicide sprays in both years, but were not influenced by N application. Disease severity can be minimized through a combination of N and fungicide applications.

Carrot cavity spot incidence and severity as affected by pigmentation and weather

M. MCDONALD (2), K. Vander Kooi (2), M. T. Tesfaendrias (2), P. Simon (1)
(1) USDA, ARS and University of Wisconsin, Madison, WI, USA; (2) University of Guelph, Guelph, ON, Canada
Phytopathology 98:S101

Cavity spot of carrot, caused by several species of Pythium, is an important soilborne disease of carrots grown in organic and mineral soils in Canada. Field trials were conducted in the Holland/Bradford Marsh region of Ontario, from 2002–2007, to determine the effect of carrot pigmentation and weather on cavity spot incidence and severity. White, yellow, red and purple carrot breeding lines from the USDA breeding program at the University of Wisconsin were seeded in organic soil (pH 6.4 – 7.2, 39–60% organic matter) in late May, harvested in late October and assessed for disease in early December, each year from 2002–2007. Commercially available cultivars with orange pigmentation were included for comparisons. Disease incidence and severity were highest in carrot cultivars with red pigmentation in five of six years, while purple pigmented carrots had the lowest incidence and severity in all years. Results indicated the influence of rainfall and temperature was greatest two and three months after seeding. Disease incidence in all the lines increased with increasing rainfall in July ($r = 0.79$), which ranged from 29 to 102 mm over the 6 years. High mean maximum air temperatures in August were correlated with low incidence ($r = –0.87$) and severity of cavity spot ($r = –0.90$), over a range of 24 to 27°C. Further confirmation of the importance of rainfall in contributing to cavity spot development, and high temperatures in reducing cavity spot, could allow a forecast of disease incidence and severity at harvest. Red pigmented carrot cultivars were very susceptible and would be useful as a susceptible check in cavity spot research.

Emergence and progression of streptomycin resistance in *Erwinia amylovora* in Michigan

G. C. MCGHEE (1), J. Guasco (1), L. M. Bellomo (1), S. Blumer (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI, USA
Phytopathology 98:S101

Streptomycin-resistant (SmR) *E. amylovora* strains were first isolated in southwest Michigan in 1991. Since that time, resistant strains have gradually progressed northward to other apple and pear-producing regions. The large majority of SmR strains harbor the *strA-strB* genes on transposon Tn*5393*. *strA* and *strB* encode phosphotransferase enzymes that modify streptomycin to a nontoxic form. Mutational resistance to streptomycin, caused by a target-site alteration of the ribosomal *rpsL* gene, occurs in a small subset of *E.*

amylovora strains from Michigan. Tn*5393* was originally introduced to *E. amylovora* on the plasmid pEa34; thus, SmR strains contained both pEa34 and the ubiquitous virulence plasmid pEA29. More recently, we have observed SmR strains in which Tn*5393* is encoded on pEA29, suggesting that the transposon has moved via transposition from pEa34 to pEA29. Because pEA29 is a nonconjugative plasmid, resistance is not thought to move horizontally through the bacterial population, and each insertion of Tn*5393* into pEA29 is viewed as a separate event characterized by the unique location of the transposon on the plasmid. This unique insertion site allows for the tracking of specific SmR strains between discrete growing regions of the state or for the observation of new SmR strains arising in these new areas. Our current data has identified two dominant transposon insertion sites on plasmid pEA29 at bps 1515 and 17527. This data suggests that a small number of SmR strains are responsible for the dissemination of streptomycin resistance in Michigan.

University of Florida Plant Medicine Program: Leading in the success of an emerging profession

R. J. MCGOVERN (6), A. J. Silagyi (4), S. M. Bledsoe (1), J. S. Strickland (5), J. A. Garces (2), C. R. Semer (7), J. Browning (3), G. N. Agrios (6)
(1) Massey Services, Inc., 3210 Clay Ave., Suite B, Orlando, FL 32804; (2) Skinner Nurseries, 13000 State Rd. 11, Bunnell, FL 32110; (3) Texas A&M University, Department of Plant Pathology and Microbiology, 4213 Chambers Lake Dr. SE, Lacey, WA 98503-3130; (4) USDA-APHIS-PPQ, Cooperative Agricultural Pest Survey, 1911 SW 34th Street, Gainesville, FL 32608; (5) University of Florida-IFAS, Hernando County Extension Office, 19490 Oliver St., Brooksville, FL 34601; (6) University of Florida-IFAS, Department of Plant Pathology and Plant Medicine Program, 1453 Fifield Hall, Gainesville, FL 32611; (7) University of Florida-IFAS, Plant Medicine Program, 1453 Fifield Hall, Gainesville, FL 32611
Phytopathology 98:S102

Human and animal health professions have evolved practitioners, physicians (MDs) and veterinarians (DVMs), to integrate and apply health information for their clients. We believe that comparably trained individuals knowledgeable in prevention, diagnosis and management of all types of plant problems, represent a natural and essential evolution of agricultural science. Since 2003, 34 American and international students have completed the rigorous, multidisciplinary training required for the Doctor of Plant Medicine (DPM) degree offered at the University of Florida (UF). Students in this program develop problem-solving skills through class room, internship and clinical trial experiences. The UF Plant Medicine Program's problem-based curriculum enables graduates to recognize and adapt to the ever-changing plant health needs of their clients. DPMs are employed as crop consultants, extension faculty, teachers, pest regulatory officials (U.S. and abroad), plant health managers (golf courses, crop production companies, public gardens, etc.) and in other segments of agriculture. Because of the increasing loss of expertise in the U.S. and elsewhere through retirement, broadly trained individuals will no doubt be in high demand to fill leadership roles in all areas of plant health management. The recognition of the emerging profession of Plant Medicine is reflected in the establishment of programs similar to University of Florida's in the U.S. and internationally.

The impact of Prohexadione-calcium and Paclobutrazol on the vascular tissue of apple

M. J. MCGRATH (1), J. M. Koczan (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI 48824
Phytopathology 98:S102

The severity of fire blight, caused by *Erwinia amylovora* (Ea), can be reduced by the shoot-growth regulators Prohexadione-calcium (ProCa) and Paclobutrazol (Paclo). However, the mechanisms of control remain unknown. To investigate the possibility that growth regulators alter the vascular tissue, ProCa or Paclo was applied to potted apple trees (cv. Gala) in 2007. Shoots were collected 0, 7, 13, and 21 d after application. Thin-sections, 0.5 and 2 cm from the leaf tips of the newest unfolded and the 2nd newest unfolded leaves, were prepared for scanning electron microscopy. Cell wall measurements of the vascular tissue (midveins) were subsequently recorded. At 21 d the ProCa-treated newest leaves, 0.5 cm from the leaf tip, had significantly ($P \leq 0.05$) thicker cell walls than Paclo- and non-treated leaves (1.5 ± 0.07, 1.3 ± 0.11, and 1.2 ± 0.08 µm respectively with S.E.). Both ProCa- and Paclo-treated leaves 2 cm from the leaf tip (21 d) had significantly thicker cell walls than non-treated leaves (1.8 ± 0.06, 1.9 ± 0.03, and 1.6 ± 0.12 µm respectively). Results from the 2nd leaves (21 d) were similar, except ProCa- and Paclo-treated leaves had significantly thicker cell walls than non-treated leaves at both 0.5 cm and 2 cm. In general, these results indicate that growth regulators can alter the vascular tissue. If thickened cell walls impede the spread of Ea throughout shoots, it could explain why severity is reduced with the application of growth regulators.

Sensitivity of *Podosphaera xanthii* to registered fungicides at-risk for resistance related to their efficacy for powdery mildew in pumpkin

M. T. MCGRATH (1), M. M. Miazzi (2)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, LIHREC, Riverhead, NY, USA; (2) Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via Amendola 165/a, 70127 Bari, Italy
Phytopathology 98:S102

A seedling fungicide sensitivity bioassay was conducted on 24 Jul 2007 at the start of powdery mildew development on Long Island, NY, in research plantings of squash and pumpkin (*Cucurbita pepo*) not treated with powdery mildew fungicides. Based on these results, the proportion of the pathogen population estimated to be resistant to QoI (FRAC Group 11) fungicides was 82%; 72% and 44% were tolerant of myclobutanil (Group 3) at 20 and 120 ppm, respectively; and 26% and 2% were tolerant of 125 and 175 ppm boscalid (Group 7). No powdery mildew developed on seedlings treated with 5 to 20 ppm quinoxyfen (Group 13). Fungicides with these active ingredients were evaluated on pumpkin in an adjacent experiment with applications applied weekly starting at the action threshold (31 Jul to 5 Sep). Quintec (quinoxyfen) was most effective, providing 81% control on lower leaf surfaces based on AUDPC values. Control was 78% with Procure (triflumizole, Group 3), 60% with Endura (boscalid), and 58% with Pristine (boscalid + pyraclostrobin). Based on a seedling bioassay conducted in this experiment on 10 Aug, 13% of the pathogen population tolerated 5 ppm quinoxyfen, 23% tolerated 175 ppm boscalid, and 11% tolerated 120 ppm myclobutanil. On 2 Oct, 9% tolerated 10 ppm quinoxyfen, 22% tolerated 200 ppm boscalid, 11% tolerated 120 ppm myclobutanil, and 2% tolerated 120 ppm triflumizole.

Long-term effects of fuel reduction treatments on the incidence of *Phytophthora* spp. in soil of a hardwood forest in the southern Appalachian Mountains

I. M. MCLAUGHLIN (1), S. N. Jeffers (1), T. A. Waldrop (2)
(1) Clemson University, Clemson, SC, USA; (2) U.S. Forest Service, Southern Research Station, Clemson, SC, USA
Phytopathology 98:S102

The accumulation of fuels is one of the main contributors to forest fires. The National Fire and Fire Surrogate study was initiated in 2000 to investigate the effects of fuel reduction treatments on a variety of ecosystem parameters in forests across the United States. We have been studying the effects of these treatments on the incidence of *Phytophthora* spp. in forest soils of the southern Appalachian Mountains. The study site was located in Polk Co. in western North Carolina and was composed primarily of hardwood and southern pine trees. Four fuel reduction treatments were applied to plots in each of three replicate blocks: prescribed burning, mechanical fuel reduction, fuel reduction followed by burning, and a non-treated control. Ten sub-plots (20 m × 50 m) were established in each treatment plot of each block. Representative, composite soil samples were systematically collected from sub-plots before treatments were applied in 2002, after one application of all treatments in 2004, and after a second application of prescribed burning in 2007. A baiting bioassay, using camellia leaf pieces and hemlock needles as baits, was used to assay soil sub-samples for *Phytophthora* spp. Only two species of *Phytophthora* were recovered throughout this investigation; *P. cinnamomi* was recovered from 33% of sub-plots in 2002, 31% of sub-plots in 2004, and 57% of sub-plots in 2007 while *P. heveae* was recovered from less than 1% of the sub-plots at each sample period. Fuel reduction treatments did not significantly affect the incidence of *Phytophthora* spp. in sub-plots over the 5-yr duration of this project. However, incidence in all sub-plots combined was greater in 2007 than in either 2002 or 2004. Overall, fuel reduction treatments did not have any immediate or long-term effects on the incidence of *Phytophthora* spp. in forest soil during this study.

***Bacillus* spp. to manage seed-born *Colletotrichum gossypii* var. *cephalosporioides* damping-off**

F. H. MEDEIROS (3), R. M. Souza (4), H. M. Ferro (4), F. C. Medeiros (3), A. W. Pomella (1), J. C. Machado (4), H. Santos Neto (4), D. A. Soares (4), E. Zanotto (4), P. W. Pare (2)
(1) Sementes Farropilha, Patos de Minas, MG, Brazil; (2) Texas Tech University, Lubbock, TX, USA; (3) Texas Tech University, Lubbock, TX, USA / UFLA, Lavras, MG, Brazil; (4) UFLA, Lavras, MG, Brazil
Phytopathology 98:S102

Damping off at seedling-stage and reduced growth associated to excessive branching (ramulose) at plant maturity are common symptoms observed in cotton with infection by *C. gossypii* var. *cephalosporioides* (Cgc). In seeking alternative biological control agents for this aggressive fungal pathogen that traditionally requires multiple fungicidal applications to control field outbreaks, over 350 *Bacillus* spp. obtained from research stocks as well as isolated from field-grown cotton roots, were screened. Seeds artificially infested with the pathogen (*Cgc*) were treated with each test strain and

seedlings rated for disease severity at three-day intervals up to 15 days after planting. Severity data were analyzed based on the area under the disease progress curve. Three strains showed consistent disease reduction (45, 57 and 59%) compared to untreated controls and outperformed *Bacillus subtilis* GB03 (the bacterial ingredient in the commercially available biocontrol product Kodiak®) (25%) and the recommended fungicide triadimenol (active ingredient of Baytan®) (35%). In field studies, a mixture of two or three strain-mixture of *Paenibacillus lentimorbus* MEN2 and *Bacillus* sp. 285 or *Bacillus subtilis* ALB629 applied to seeds resulted in greater disease protection against damping off than any of the strains applied individually or the commercial fungicide. Effects of these benefitial *Bacillus* spp. on seed germination and growth promotion are also presented. Therefore, to reduce fungicide loads and fungal resistance, the bacterium mixture (*P. lentimorbus* MEN2 and *Bacillus* sp. 285) provides a plausible alternative for pathogen control in cotton.

Study of the progression of a cotton seed and boll bacterial infection resulting from vectoring of the phytopathogen by the southern green stink bug (*Nezara viridula* L.)

E. G. MEDRANO (1), J. F. Esquivel (1), A. A. Bell (1)
(1) USDA-ARS, College Station, TX, USA
Phytopathology 98:S103

Background: Previously, we determined that the southern green stink bug (SGSB) with a piercing/sucking mode of feeding can transmit an opportunistic strain of *Pantoea agglomerans* that was originally isolated from a diseased South Carolina cotton boll. Here, we describe effects of boll age on the *P. agglomerans* infection process. *Methods:* A lab-reared colony of SGSB fed fresh green beans was used in the transmission studies. Greenhouse-grown bolls at 2 or 3 wks post-anthesis (WPA) were individually caged for 2 days either with no stink bug, an adult without a pathogen, or an adult that harbored a cotton pathogen. Infestation of the SGSB with the *P. agglomerans* rifampicin (Rif) resistant strain Sc 1-R involved dipping a bean in a pathogen suspension before providing it to the insects. Bolls were either harvested 3 wks after being caged or grown to yield. Locule tissue from unopened bolls were aseptically collected, triturated, and then plated on Luria-Bertani agar (LBA) or LBA amended with Rif. *Results:* Bacteria were recovered on LBA at levels reaching 10^9 CFU/g tissue from all bolls with evidence of insect feeding. However, disease symptoms were only present on samples caged with insects harboring the Sc 1-R pathogen. Concentrations of Sc 1-R from bolls exposed at 2 WPA reached 10^8 CFU/g tissue with the entire locule rotted. Conversely, Sc 1-R levels reached 10^3 CFU/g tissue from fruit caged at 3 WPA and a yellow fiber discoloration occurred only around the point of insect puncture. Locule tissue of cracked bolls from fruit exposed at 2 WPA was dark, matted and dense. Fiber from opened control bolls and fruit exposed to Sc 1-R at 3 WPA were asymptomatic. *Conclusion:* The vulnerability of bolls to SGSB infestation and/or a vectored pathogen infection decreased with the increased maturity of the boll at the time of the challenge.

Detection of *Agrobacterium tumefaciens* in soil

E. MEEKES (2), R. Butôt (2), S. Lieten (1), B. Geurkink (1), I. Dinkla (1)
(1) Bioclear, Groningen, the Netherlands; (2) Naktuinbouw, Roelofarendsveen, the Netherlands
Phytopathology 98:S103

Crown gall disease, caused by *Agrobacterium tumefaciens*, causes significant economical loss in propagation material of rose and fruit trees. Infection by *A. tumefaciens* can occur during grafting, watering or when clean propagation material is planted on fields with *A. tumefaciens*. Up to now, producers of root stock of *Rosaceae* could only determine infection at the end of the cultivation when lifted plants showed visual symptoms. Therefore a PCR-method was developed, based on the tumour-inducing plasmid, to detect pathogenic cells of *A. tumefaciens* biovar I and II in soil before planting. Fields with a history of *A. tumefaciens* were sampled several times during two years, the last year using different subplot sizes. After two years of fallow and/or cultivation of non-host plants, *A. tumefaciens* could still be detected. The relation between results of the analyses, crop history and percentages of the plants with symptoms will be discussed. The PCR-based method will enable growers to make an informed decision when selecting fields for propagation material and thereby reduce the risk of infection by *A. tumefaciens*.

The current status of chrysanthemum white rust in the United States

F. MEHDIZADEGAN (1), S. Redlin (1)
(1) USDA, PPQ, Raleigh, NC, USA
Phytopathology 98:S103

Puccina horiana P. Henn., the causal agent of chrysanthemum white rust (CWR), is known to occur in many countries but it is eradicated by Federal and state cooperators anywhere it is detected within the United States. The purpose of this report is to identify pathways and potential mitigation strategies to minimize the risk of introduction and establishment. *P. horiana* was first detected and eradicated in the U.S. in 1977 in New York and later in Pennsylvania (1978), Oregon and Washington (1990), and California (1991). Since 1995, CWR has been occasionally detected in regional surveys and eradicated. In 2007, CWR was found in a number of retail establishments in the eastern U.S. (CT, DE, GA, MD, ME, NC, RI, SC, PA, and VA). Potential means of spread include imported and domestic cut flowers, florist mums, and garden mums. Risk analyses conducted by PPQ concluded that the probability of introduction is low for cut flowers and medium for some florist and garden mums, depending on region and season. Measures for reducing the probability of introduction include: 1) developing an integrated management program at the origin for growing CWR-free mums and 2) closer monitoring of nurseries at the origin and destination. Additional research is needed to determine the origin of seasonal outbreaks. Consideration may also be given to deregulation of the pathogen as a quarantine pest for the U.S. based on low impact and ease of management of the disease.

Variability in competitive ability among *Aspergillus flavus* vegetative compatibility groups during maize infection

H. L. MEHL (1), P. J. Cotty (1)
(1) USDA-ARS, Dept. Plant Sciences, University of Arizona, Tucson, AZ, USA
Phytopathology 98:S103

Aflatoxin, a carcinogenic toxin produced by several *Aspergillus* spp., is a frequent contaminant of food and feed crops; vegetative compatibility groups (VCGs) of *A. flavus* differ in the ability to produce aflatoxins. The current study sought to quantify competitive differences among VCGs during infection of living maize kernels. Seed (5 g) were inoculated with one of 40 different VCGs followed by an equal amount of a common aflatoxin-producing VCG (VCG U) and incubated at 31°C for 7 days. Spores and seed were subsequently separated and aflatoxin and DNA were extracted from each. Aflatoxin B_1 was quantified with florescence densitometry on thin layer chromatography plates. In order to assess VCG dominance in seed and spores, VCG-specific SNPs were quantified by pyrosequencing. The 40 VCGs varied in competitiveness against VCG U with U comprising 17–55% and 18–51% of the total *A. flavus* DNA in seed and spores, respectively. The relative amounts of seed infection and sporulation were not strongly correlated (r^2 = 0.25, $P < 0.0001$) indicating that sporulation on crop surfaces does not necessarily reflect relative crop infection. Compared to maize inoculated with only U, toxin was reduced up to 91% in co-inoculated seed and 94% in spores from that seed. Improved understanding of competition among *A. flavus* VCGs during crop infection will facilitate efforts to reduce aflatoxin contamination through competitive exclusion of aflatoxin producers.

Isolation and characterization of two xylanases from *Fusarium graminearum*

S. W. MEINHARDT (1), X. Dong (2), P. B. Schwarz (2)
(1) Dept. of Plant Pathology, North Dakota State University; (2) Dept. of Plant Sciences, North Dakota State University
Phytopathology 98:S103

Fusarium graminearum (teleomorph *Gibberella zeae*), causes Fusarium Head Blight (FHB) of wheat and barley, resulting in severe yield losses and crop quality reductions. Although *F. graminearum* produces multiple toxins, the cell wall degrading enzymes may be more important in pathogenesis. We have purified two xylanases from *F. graminearum* 52- and 40-fold using four chromatographic methods: ion-exchange, gel filtration, HPLC ion-exchange and HPLC hydrophobic interaction chromatography. SDS-PAGE gave a single band for each enzyme with estimated masses of 20 and 40 KDa. Trypsin digestion and LC-MS/MS identified the two xylanases as the gene products of FG03624 (62% coverage, 20 kDa) and FG06445 (87% coverage, 40 kDa). Xylanase FG06445 exhibited a broad pH optima, from pH 5.5 to 8.5, but dropped rapidly above pH 8.5 and below pH 5.5. FG03624 was active from pH 5.5 to 7.5. Both enzymes were stable at pH 5.5 and 8.5, losing only 20% of the activity over 5h at room temperature. The activity of xylanase FG06445 was optimal at 50C and FG03624 was at 45C. The low molecular weight xylanase activity changed little over 5h at 35C while the high molecular weight xylanase lost up to 40% of its activity.

The occurrence of a distinct variant of *Grapevine fanleaf virus* in Washington State vineyards

T. A. MEKURIA (1), R. R. Martin (2), N. A. Rayapati (1)
(1) Department of Plant Pathology, Washington State University, Irrigated Agriculture Research and Extension Center, Prosser, WA; (2) USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330
Phytopathology 98:S103

Grapevine fanleaf virus (GFLV, genus: *Nepovirus*, family: *Comoviridae*), is one of the most important viral diseases of wine grapes (*Vitis vinfera*) world-wide. During our reconnaissance studies, samples from the wine grape cultivar Chardonnay collected in two separate vineyards tested positive for GFLV in RT-PCR using primers specific to the coat protein. Sequence analysis of the 322 base pair amplicon showed 89–91% identity with corresponding

sequences of GFLV isolates in GenBank. Additional analysis by ELISA was positive for GFLV antibodies. These results confirmed the presence of GFLV in vineyards in Washington. The virus was found as mixed infection with *Grapevine leafroll-associated virus* (GLRaV)-3 in one vineyard and with GLRaV-1, GLRaV-3 and *Grapevine virus A* in the other vineyard. The RNA2 from one GFLV isolate was characterized by cloning and sequencing RT-PCR amplified DNA fragments. The cloned fragments covered 3564 nucleotides (nt) of the RNA2 and showed 90% sequence identity with GFLV strain F13 from France. The genetic organization of RNA2 revealed a single polyprotein of 1190 amino acids with 90% sequence identity to the corresponding sequence of GFLV isolates from different parts of the world. The three RNA2-encoding proteins showed 74–79% ($2A^{HP}$), 88–90% ($2B^{MP}$) and 88–90% ($2C^{CP}$) identity at the nucleotide level and 75–78% ($2A^{HP}$), 95–96 ($2B^{MP}$) and 94–96% ($2C^{CP}$), at the amino acid level with corresponding sequences of GFLV isolates from different parts of the world. These results indicate the presence of a distinct GFLV isolate in Washington State vineyards. To our knowledge, this is the first report of GFLV in the Pacific Northwest region.

Citrus stubborn symptom severity and *Spiroplasma citri* location within the tree canopy

A. S. MELLO (2), R. K. Yokomi (1), M. Payton (2), J. Fletcher (2)
(1) ARS-USDA, Parlier, CA, USA; (2) Oklahoma State University, Stillwater, OK, USA
Phytopathology 98:S104

The severity of symptoms of citrus stubborn disease (CSD) within an orchard can range from mild to severe, but whether factors other than pathogen titer or duration of infection impact severity is not known. We tested the hypothesis that the canopy distribution of the pathogen, *Spiroplasma citri*, is related to symptom severity. When fruits were harvested randomly from the canopies of trees from a commercial orchard and *S. citri* presence assessed by culturing from fruit receptacles, a greater percentage of samples from severely symptomatic trees yielded cultures, and bacteria grew to log phase more quickly, than from mildly symptomatic trees. In a second experiment, both fruits and leaves from two canopy aspects (east and west) and three canopy tiers (top, middle, and base) were subjected to q-PCR, using P58 gene-based primers, on DNA from fruit columellas and leaf petioles. The percentage of samples testing PCR-positive was significantly greater from severely symptomatic than from mildly symptomatic trees, and columellas yielded more q-PCR positives than did petioles. However, neither canopy aspect (east vs west) nor tier (upper, middle or lower) correlated significantly with percentage *S. citri* detection. The data suggest that, within this orchard, CSD severity is unrelated to the overall within-tree pathogen distribution, but is correlated with the percentage of samples containing the pathogen.

Endospore-forming bacterial endophytes of cacao: Ecology and biological control of witches' broom

R. L. MELNICK (3), C. Suárez (1), D. I. Vera (1), B. A. Bailey (2), P. A. Backman (3)
(1) Instituto Nacional de Investigaciones Agropecuarias, Estación Experimental Tropical Pichilingue, Quevedo, Los Rios, Ecuador; (2) SPCL, USDA-ARS, Beltsville, MD, USA; (3) The Pennsylvania State University, University Park, PA, USA
Phytopathology 98:S104

Interest in ecologically-based sustainable farming has increased research on biological control for management of cacao diseases in South and Central America. Endospore-forming bacterial endophytes were isolated from superior cacao trees near Quevedo, Ecuador and screened as potential biological control agents for cacao diseases. Four elite *Bacillus* spp. are currently being field evaluated to determine their ability to suppress witches' broom disease. Bacteria were spray applied in May at log 8.0 CFU/ml with 0.20% Silwet L-77 to four clonal varieties with varying susceptibilities to witches' broom. The bacteria survived in the foliage throughout the duration of the dry season (May through November). Colonization of foliage did not significantly suppress disease during the dry season, due to low overall disease incidence. Although significant suppression did not occur, there was a strong trend of disease reduction by isolate CCAT1858 2.1.2. Bacteria were reapplied to the foliage in December to evaluate treatments during the rainy season. An additional field experiment was designed to evaluate these same biocontrol agents on branches and pods of established trees. Data will be presented on both of these experiments and the effects of treatments on native microbial populations of the cacao phyllosphere.

Effect of water potential on sclerotial production by *Sclerotinia sclerotiorum* in a culture medium

H. MELOUK (1)
(1) USDA-ARS, ENTO & PLP, OK State University, 127 NRC, Stillwater, OK 74078
Phytopathology 98:S104

Sclerotinia sclerotiorum causes Sclerotinia blight on peanut. Potato dextrose agar medium was prepared and adjusted to various water potentials (–0.4 to –3.4 MPa) using NaCl. Petri plates (9-cm dia) each containing 15 ml of medium were inoculated with a 4-mm agar plug of *S. sclerotiorum*. Plates were incubated at 25°C in darkness for 3 weeks, and then were allowed to dry by un-covering the plates for 2 weeks at 24 + 2 C. Sclerotia were removed from the semi-dry medium by using a camel hair brush. Sclerotial mass (g) from each plate was determined. Sclerotial weights from cultures grown at –0.9, –1.4, – 1.9, –2.4, and –3.4 MPa were significantly ($P = 0.05$) increased from 3 to 8 fold when compared with those grown at –0.4 MPa. Data from this study suggest that water stress enhances the ability of *S. sclerotiorum* to produce more sclerotia for its survival.

The worldwide occurrence of the anther-smut fungus *Microbotryum* on species of the Caryophyllaceae as assessed from herbarium surveys

J. MENA-ALI (1)
(1) Dept. of Biology, Amherst College
Phytopathology 98:S104

Invasion/infection of pathogens onto a new host can be affected by various traits of the host that limit the pathogen's survival, reproduction and spread to more individuals in the population. The effect of these traits will in part depend on the life history of the pathogen, as well as its specificity and virulence. *Microbotryum violaceum* causes anther-smut disease in members of the Caryophyllaceae. Obligate pathogens with very restricting life cycles such as *M. violaceum* are expected to preferentially infect perennial host species. Here we greatly expand this hypothesis by surveying over 28 thousand herbarium specimens of Silene and allied genera of the Caryophyllaceae to determine the worldwide distribution of *M. violaceum*. These data was then examined for associations between the probability of infection and life history of the host, floral morphology, and breeding system. Our analyses expand the assumed range of the pathogen from an exclusively temperate Northern distribution to a worldwide presence, to include subtropical locations. These results are discussed in light of host distribution over a broad geographic range and the role of potential host-shifts in dispersal at an inter-continental scale.

Interactions with hosts at cool temperature, not cold tolerance, explain the unique epidemiology of *Ralstonia solanacearum* Race 3 biovar 2

F. MENG (1), A. Milling (1), C. Allen (1)
(1) Dept. Plant Patholoygy, UW-Madison, Madison, WI
Phytopathology 98:S104

Most strains of the bacterial wilt pathogen *Ralstonia solanacearum* are tropical, but one group, R3bv2, can attack plants in temperate zones and highland tropics. Extensive epidemiological data document the destructiveness of R3bv2 in cooler climates, but the basis of this distinctive ecological trait is not understood. We compared the survival, growth and virulence of two *R. solanacearum* strains, GMI1000 (race 1, tropical) and UW551 (R3bv2, temperate) at different temperatures with and without host plants. At 4C (the temperature of commercial seed potato storage), neither GMI1000 nor UW551 survived more than 90 days in water. However, UW551 survived more than 4 months in inoculated potato tubers at 4C, while GMI1000 survived less than 50 days in tubers. The two strains grew similarly in minimal media at 20C and 29C. At 29C, both strains wilted tomato plants rapidly in a naturalistic soil-soak virulence assay. In contrast, at 20C UW551 was much more virulent on tomato than GMI1000. Thus, there was little difference in growth and survival of tropical and R3bv2 strains at 4C, 20C, or 29C in the absence of a plant host. But at cooler temperatures R3bv2 survived longer in tubers and caused disease better than a tropical strain. These data indicate interaction with plants is required for the temperate epidemiological trait of R3bv2.

Spatio-temporal dynamics of black leaf mold (*Pseudocercospora fuligena*) across the tomato canopy in natural and artificial epidemics under protected cultivation in Thailand

Z. MERSHA (1), B. Hau (2)
(1) Leibniz Universitaet Hannover; (2) Leibniz Universitaet Hannover, Institute of Plant Disease and Plant Protection, Herrenhauser Str. 2, 30419, Hannover, Germany
Phytopathology 98:S104

The vertical distribution of black leaf mold (BLM), caused by *Pseudocercospora fuligena*, was investigated across the canopy of the tomato cultivar FMTT260 under protected cultivation in Thailand. After 16 weeks of a natural epidemic, BLM severity (*DS*) of the lower canopy layer (0–50 cm) was 42% and significantly higher compared to 26% of the middle (51–150 cm) and 5% of the upper (>150 cm) layer. During the growing period of another natural epidemic, a similar distribution of BLM was observed, but higher *DS* and bigger lesions were detected on leaf positions 5 to 10 (in

ascending order from bottom up) than the first four leaves borne in the nursery. Further non-destructive samplings until 65 days after transplanting revealed the earliest BLM symptoms on leaf positions 5 to 8. When cohorts of 5 leaves were formed starting from the bottom, BLM incidence of leaves of the 1st cohort was only 79% while the next two cohorts reached 100%. In artificially inoculated plants, however, BLM was clearly more prevalent in the middle layer of the plant canopy as compared to the lower and the top part. Plants inoculated at 4 weeks after transplanting and monitored 10 days later, showed a *DS* of 43, 67 and 21% on the 1st, 2nd and 3rd cohorts, respectively. Thus, given equal chance of *P. fuligena* inoculum to infect all the leaves of a tomato plant at one time, more BLM developed on fully expanded younger leaves than older once. High BLM severity of the lower canopy is not related to the age of tomato leaves, but attributed to proximity to substrate evaporation coupled with the down-hanging nature of FMTT260 leaves that created a confounding microclimate which led to higher relative humidity within the range of about 50–70 cm.

Increased severity of fungal foliar diseases on sweet corn infected with maize dwarf mosaic

M. D. MEYER (1), J. K. Pataky (1)
(1) University of Illinois
Phytopathology 98:S105

The effect of maize dwarf mosaic (MDM) on severity of fungal leaf diseases of sweet corn was tested in field trials in 2007. Two viral treatments and 32 hybrids were included in four replicates of a split-plot, randomized complete block design. Plants at the 3- to 4-leaf stage were inoculated with *Maize dwarf mosaic virus* strain A and *Sugarcane mosaic virus* strain B or left untreated. Following viral inoculations, all plants were inoculated with fungal pathogens. The experiment was repeated individually for five diseases: southern leaf blight (SCLB), gray leaf spot (GLS), northern leaf spot (NCLS), northern leaf blight (NCLB), and common rust. Three weeks after anthesis, foliar diseases were visually assessed on a plot basis using a 1–9 scale or percent severity. Also, four leaves were sub-sampled from each plot and leaf area infected (LAI) was assessed with imaging software. In the ANOVAs, the hybrid, viral treatment, and hybrid × treatment interaction terms were significant. For SCLB, NCLS, and GLS, severity and LAI were significantly and substantially greater on virus-inoculated plants than on non-inoculated plants. Main effect means of LAI for SCLB, NCLS, and GLS were 23%, 38%, and 28%, respectively, from virus-inoculated plants and 6%, 11%, and 6% from non-inoculated plants. For NCLB and rust, severity and LAI were significantly but not appreciably greater on virus-inoculated plants than on non-inoculated plants. Main effect means of severity for NCLB and rust were 38% and 34%, respectively, from virus-inoculated plants and 35% and 28% from non-inoculated plants. For each foliar disease, severity and LAI were not significantly different between virus-inoculated and non-inoculated plants of five MDM-resistant hybrids.

Suppression of *Meloidogyne incognita* population densities with DIBOA

S. L. MEYER (2), I. A. Zasada (2), C. Rice (1)
(1) USDA ARS Environmental Management and Byproduct Utilization Lab, Beltsville, MD, USA; (2) USDA ARS Nematology Lab, Beltsville, MD, USA
Phytopathology 98:S105

Benzoxazinoids produced by rye (*Secale cereale*) can be nematotoxic. To test effects of one of these compounds on nematode population densities, the benzoxazinoid 2,4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one (DIBOA) was added to soil at concentrations ranging from 9.4 to 150 micrograms per ml (dissolved in methanol and water). DIBOA-treated and control soils were inoculated with *Meloidogyne incognita* and incubated for five days, during which time the soil was sampled at intervals to investigate disappearance of DIBOA and formation of BOA and HBOA, two important metabolites or degradation products of DIBOA. The soil treatments were then planted with cucumber seedlings and grown in the greenhouse for 5 weeks. Disappearance half-life values for DIBOA varied from 14.6 to 24.1 hours. Production of BOA and HBOA were approximately equal and their combined total concentrations never exceeded 10% of the initial DIBOA concentrations. After their initial formation these metabolites also disappeared over the course of the sampling time. Only the highest DIBOA treatment suppressed nematode populations on cucumber roots, with populations approximately 73% of those recorded from the water plus methanol controls. This result was different from the effects previously observed in laboratory assays, in which a lower concentration of DIBOA, 74.3 micrograms per ml, had been sufficient to prevent 50% of eggs from hatching. The fate of DIBOA in soil may account for differences between effects of DIBOA in laboratory assays vs. greenhouse tests.

Soybean root colonization by two Fusarium species is determined by soil moisture

P. W. MEYER (1), J. L. Colburn (1), J. E. Kurle (1)
(1) Dept. of Plant Pathology - University of Minnesota
Phytopathology 98:S105

Seed, seedling, and root rots of soybean are the most important fungal diseases of soybean in Minnesota. The result of infection by a complex of soilborne fungi, they cause stand reduction, root damage, and poor plant growth with yield losses estimated at 380,000 tons in 2005. The interaction of soil temperature and moisture in infection by these pathogens is poorly understood. In a previous study, we found that at 2 weeks after planting, Fusarium spp. preferentially infect soybean at 90°F, in either saturated soil watered daily and maintained above 3kPa, or in dry soil watered every 7 days and allowed to dry to below 100kPa matric potential. Soybean plants grown in naturally infested soil for 14 days were sampled for infection by *Fusarium*, *Rhizoctonia*, and *Pythium* spp. and *Phytophthora sojae*. Taproots were incubated on selective media and probable *Fusarium* colonies identified by morphology and growth rate. *Fusarium* isolates were identified to genus level by shape and size of macroconidia. Isolates were then identified to species level by ITS region sequencing and BLAST search on GenBank. We found that *F. solani* infected plants grown in either saturated or extremely dry soil, while *F. oxysporum* was isolated only from plants grown in saturated soil. We hypothesize that the infection optimum in dry soil is due to plant stress. Research is needed to determine why *F. solani* infects plants under either saturated or dry conditions, while *F. oxysporum* does not.

Selective accumulation of *Trichoderma* spp. in soils suppressive to radish damping-off disease

J. MGHALU (2), M. Kubota (1), M. Hyakumachi (1)
(1) Gifu University, Gifu, Japan; (2) Pwani University College, Kilifi, Kenya
Phytopathology 98:S105

Disease decline in radish damping-off was artificially induced through repeated soil inoculations with *Rhizoctonia solani*, binucleate *Rhizoctonia* and *Sclerotium rolfsii* in pot systems. Species of *Trichoderma* were consistently isolated from the inoculated soils showing disease decline. Populations of different *Trichoderma* spp. accumulated selectively in relation to the soil pathogen inoculated. Occurrence frequencies of *T. viride*, *T. harzianum* and *T. hamatum* were 3:1:1 and 3:2:1 in soils inoculated with *R. solani* and binucleate *Rhizoctonia*, respectively. In *S. rolfsii* inoculated soils, *T. koningii* was predominantly isolated. Isolates of *T. viride*, *T. harzianum* and *T. hamatum* obtained from both *R. solani* and binucleate *Rhizoctonia* repeatedly inoculated soils suppressed radish damping off disease caused by *R. solani* and *S. rolfsii* at varied levels. However, isolates of *T. koningii* obtained from *S. rolfsii* inoculated soils, suppressed disease caused by *S. rolfsii* but failed to suppress disease caused by *R. solani*. The *Trichoderma* species accumulated in a selective pattern that was correlated to the soil pathogen inoculated to induce the disease suppressive soils.

Infection of pomegranate by *Alternaria* spp. causing black heart

T. MICHAILIDES (2), D. Morgan (2), M. Quist (1), H. Reyes (2)
(1) Paramount Farming Company, Lost Hills, CA, USA; (2) University of California-Davis, Kearney Agricultural Center, Parlier, CA, USA
Phytopathology 98:S105

After the discovery of high levels of antioxidants in pomegranates, the acreage of pomegranates grown for fresh and juice consumption has increased significantly in California, to about 20,000 acres presently. A major concern of pomegranate growers is a disease called black heart, characterized by decay of arils by black fungi with damage ranging from sections of the fruit to all the arils within the exocarp. The fungus that was most frequently isolated from these symptomatic fruit was *Alternaria alternata* and other *Alternaria* spp. A second pathogen that was also isolated alone or in combination with *Alternaria* in up to 15% of the cases was *Aspergillus niger*. To determine the time of infection, serial inoculations, starting at bloom and continuing until the end of June were conducted by spraying flowers and developing fruit with a 2×10^4 spores/ml suspension of two *A. alternata* isolates (2A02 and 2B11). To create conditions favorable for infection, inoculated plant parts were covered with a plastic bag from the time of inoculation until the next morning and the fruit remained on the trees until harvest. Regardless of the *Alternaria* isolate used, the highest incidence of black heart developed in fruit inoculated in the spring (in May) at the anther dehiscence stage, suggesting that most of the infections by *Alternaria* occur at bloom time. Disease incidence declined from inoculations performed after May. In a second experiment, injections of maturing fruit with the spore suspension of the above *Alternaria* isolates resulted in more than 85% fruit with black heart while only 13.5% of the noninoculated fruit developed black heart due to natural infection. These results can help pomegranate growers in California design disease management approaches.

Quantitative real-time PCR to differentiate infection levels of *Aspergillus flavus* in maize

S. X. MIDEROS (1), W. P. Williams (2), R. J. Nelson (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY; (2) USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS
Phytopathology 98:S106

Aspergillus flavus causes Aspergillus ear rot of maize and produces aflatoxins. These potent toxins cause liver cancer, nutritional interference and immunosuppression when consumed by humans or animals. As a result, the strict regulation on the commerce of contaminated maize causes economic burdens on farmers in developed countries. In developing countries, where regulations are nonexistent or unenforced, people may be widely exposed to this toxin. There are published assertions that resistance to aflatoxin accumulation and pathogen colonization are distinct traits in maize. *A. flavus* is an aggressive saprobe for which the levels of colonization are difficult to characterize. For this reason, we have developed quantitative PCR assays to measure the levels of colonization in maize tissues. Our preliminary results show an unexpectedly high correlation between fungal load and aflatoxin levels in ground maize kernels. We will next determine whether this high correlation is confirmed in studies with more diverse maize lines and in mapping populations. Our research could have a direct impact on breeding programs that aim to identify lines with resistance to aflatoxin accumulation, and sets the stage for future studies on the genetic dissection of aflatoxin-related traits.

Investigating the molecular mechanisms of resistance to anthracnose fruit rot in blueberries

T. D. Miles (1), B. Day (1), A. C. SCHILDER (1)
(1) Department of Plant Pathology, Michigan State University, East Lansing, MI, USA
Phytopathology 98:S106

Anthracnose fruit rot, caused by the fungus *Colletotrichum acutatum*, is a major disease of blueberries and responsible for substantial pre- and postharvest losses worldwide. Most blueberry cultivars are susceptible to this disease; however, cultivar Elliott, which is widely grown in Michigan, has a lower rot incidence and reduced sporulation on the fruit. Previous microscopy studies revealed a build-up of phenolic compounds following infection in 'Elliott' but not in the susceptible cultivar Jersey, suggesting an active resistance response. In addition, the infection strategies of the fungus appeared different in the resistant versus susceptible cultivar. Few studies have been done on molecular host-pathogen interactions in fruit crops and in perennial crops in general. The primary goal of this research is to gain insight in the molecular mechanisms which underlie host resistance to anthracnose fruit rot in highbush blueberries. Using subtractive hybridization, we have identified several expressed sequence tags (ESTs) that are differentially expressed in 'Elliott' versus 'Jersey' after infection. The ESTs have been assigned a hypothetical function based on homology with known proteins and have been further characterized by creating temporal profiles of the candidate genes using RT-PCR. Some of these are known host defense genes, such as chitinase, PR10a, and beta-1-3 glucanase. Many of the candidate genes were expressed earlier in the infection process in the resistant cultivar than in the susceptible one. Increasing our understanding of the basis of resistance to *C. acutatum* by identifying potential candidate resistance genes will facilitate the development of new resistant cultivars.

Pathogenicity testing of *Agrobacterium tumefaciens* and *Rhodococcus fascians* isolates on micropropagated plants

M. L. MILLER (1), M. L. Putnam (1)
(1) Oregon State University, Corvallis, OR 97331-2902, USA
Phytopathology 98:S106

Inoculations to indicator plants of putative pathogens require a ready supply of susceptible plants and ample greenhouse space. Results may be compromised by unintentional spread of the bacterial isolates between plants by insects or water splash. Agrobacteria isolates from cherry, apple and Himalayan blackberry were inoculated to these hosts *in vitro*. Symptoms on blackberry were observed within 1 week and by 2 weeks, 82% of the blackberry wound sites had tumors as compared to 39% for cherry and 4% for apple. To test blackberry as an indicator, inoculations were made with virulent and avirulent agrobacteria from a wide variety of hosts. Plants were wounded with a 27 gauge needle first dipped into bacteria grown for 2 days on Mannitol Glutamate medium. After 5 weeks, tumors could be detected on plants inoculated with 58 of the 69 strains (84%); 18 avirulent strains produced no tumors. In other systems, inoculations with 5 *A. tumefaciens* strains on *Bryophyllum diagremontianum* produced symptoms within 1 week as did 5 *R. fascians* on *Oenothera speciosa. A. tumefaciens* and *A. vitus* produced tumors on grape within 2 weeks. The same plant hosts inoculated in the greenhouse develop symptoms more slowly, usually in 4 to 8 weeks. Micropropagated plants are gnotobiotic, take up little space, are easy to propagate and inoculate, develop symptoms rapidly, and offer an underutilized alternative to greenhouse testing.

Phylogenetic analysis of the ITS1 and ITS2 rDNA regions of Lycoperdaceae associated with fairy rings on golf putting greens

G. L. MILLER (1), L. P. Tredway (1)
(1) North Carolina State University
Phytopathology 98:S106

Identification of fungi associated with fairy rings is based solely on morphology of basidiocarps, which often do not develop or do not reach maturity due to turf management practices. Genomic DNA was extracted from puffballs and mycelial cords collected from 5 bermudagrass and 4 bentgrass greens in FL, HI, NC, and SC exhibiting fairy ring symptoms. The ITS1 and ITS2 regions of rDNA were amplified and sequenced using the basidiomycete-specific primer set ITS1f/ITS4b. Phylogenetic analyses, including construction of a parsimony tree and Bremer partition support calculations, were performed on the two ITS regions of 24 field-collected isolates and 6 downloaded sequences from Genbank. Parsimony analysis revealed only one tree, having high bootstrap and Bremer supports at each node. Twenty-three of the unknown puffball isolates clustered with Genbank accessions of either *Vascellum pratense* or *Lycoperdon pusillum*, not with *Lycoperdon perlatum*, which is often cited as a prevalent cause of fairy rings in golf course putting greens in the Southeast. One of the unknown isolates grouped in a separate haplotype from all others. Morphology of the gleba of five mature puffballs was also probative of fungal genera. Puffballs with a true capillitium had ITS sequences most similar to *Lycoperdon pusillum*, while puffballs without capillitial threads had sequences most similar to *Vascellum pratense*.

Effect of germicidal light on seed-borne *Fusarium* and sweet corn seedling vigor

N. MILLER (1), C. M. Ocamb (1)
(1) Oregon State University
Phytopathology 98:S106

Several species of *Fusarium* known to cause root, stalk and ear rot are commonly found on corn seed. Fungicide seed treatments suppress Fusarium growth but don't always prevent seed-borne *Fusarium* from infecting plants grown from contaminated seed. Germicidal lights are relatively inexpensive and readily available for sterilizing air and flat surfaces. The objective of this study was to determine if germicidal lights could be used to reduce the proportion of sweet corn seeds infested with *Fusarium*. Seed of nine sweet corn lines were exposed to UV-C emitting germicidal lighting while being agitated for 0, 30, 60, 120, 180, 240, or 300 min and then assayed for the presence of *Fusarium* or other fungi. Seeds were also grown for 10 days in sterile germination paper to assess viability and seedling vigor. The mean proportion of seeds yielding *Fusarium* species decreased as time under the germicidal light increased and means were significantly lower than the control for all exposure intervals except the 30 min set. Plants grown from UV-C treated seed at all time points had significantly greater root and shoot weights compared to the non-UV-C controls. Exposure of 60 min resulted in the highest mean root weight while the highest mean shoot weight was found in the 120 min sets. No differences in germination were detected. Germicidal light treatment can reduce *Fusarium* contamination on sweet corn seed and appears to enhance seedling vigor. Dry seeds could be exposed to germicidal lighting prior to treatment with fungicides or beneficial microbes, and studies are currently underway to evaluate several of these combinations.

How do plants defend themselves against bacterial wilt? Response of resistant and susceptible tomato plants to infection by *Ralstonia solanacearum*

A. MILLING (1), C. Allen (1)
(1) University of Wisconsin, Department of Plant Pathology, 1630 Linden Dr., Madison, WI 53706
Phytopathology 98:S106

Host resistance is the only practical control for bacterial wilt of tomato, caused by diverse strains of *Ralstonia solanacearum*, but wilt resistance is horizontal, polygenic and its basis is not understood. We used quantitative PCR to measure induction of six defense related genes in resistant or susceptible tomato plants following infection with either a tropical Race 1 strain or a temperate Race 3 strain of *R. solanacearum*. Tomato plants responded to infection by upregulating marker genes for both the salicylate (SA) and ethylene (ET) signaling pathways, but not the jasmonate (JA) pathway. Both host and pathogen genotype played a critical role in the tomato response to bacterial wilt. The horizontally resistant tomato line H7996 showed a faster and greater induction of the ET and SA signaling pathways than a susceptible cultivar in response to infection by either the tropical or the

temperate strain. The susceptible tomato also launched strong defenses following infection by the tropical strain, but the temperate Race 3 strain triggered little defense response in the susceptible host while causing similar symptoms. To explore mechanisms by which the pathogen may evades host recognition and resistance, we also measured defense expression in resistant and susceptible tomato plants infected with *R. solanacearum* mutants that lack either extracellular polysaccharide production or a functional Type III secretion system.

Transgenic expression of an inducible *Beet curly top virus* C4 gene leads to prolific cell division and abnormal apical development in *Arabidopsis thaliana*

K. MILLS LUJAN (1), C. M. Deom (1)
(1) Department of Plant Pathology, University of Georgia, Athens, GA
Phytopathology 98:S107

The C4 gene of *Beet curly top virus* (BCTV; *Curtovirus*, Geminiviridae) induces hyperplasia in infected tissues and induces cell division when expressed ectopically. To investigate *in planta* the role this gene has on plant development, we generated transgenic *Arabidopsis thaliana* plants in which the BCTV C4 transgene was expressed under the control of an inducible promoter (line ipC4-28). Seedlings of line ipC4-28 germinated on induction media had a severely stunted phenotype, with development arresting early in seedling germination. Seedlings expressing the C4 transgene did not develop true leaves or lateral roots, but instead showed abnormal thickening of the cotyledon and hypocotyl, whereas control lines had a wild-type phenotype. The severity of the phenotype correlated with the concentration of inducer, and thus, the amount of C4 protein expressed. The cotyledons of induced ipC4-28 seedlings developed abnormally, had a higher number of cells, and lacked normal tissue layer organization as well as a clearly defined vascular system, when compared to control lines. In addition, both the root and shoot apical meristems appeared to be non-functional as early as 3 days post germination. Furthermore, the development of specialized epidermis cell types, such as stomata and root hairs was also absent or impaired in seedlings that expressed C4 protein. Taken together these results indicate that the expression of the BCTV C4 protein in *A. thaliana* leads to abnormal loss of meristematic function, uncontrolled cell division, loss of tissue/cell layer organization, and loss of cell differentiation.

Increased aggressiveness of *Puccinia striiformis* f. sp. *tritici* at least partially explains recent stripe rust epidemics

E. A. MILUS (3), K. Kristensen (2), M. S. Hovmoller (1)
(1) University of Aarhus, Flakkebjerg, Denmark; (2) University of Aarhus, Foulum, Denmark; (3) University of Arkansas, Fayetteville, AR, USA
Phytopathology 98:S107

Stripe rust (yellow rust) of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, has become more severe since 2000 in eastern United States, Australia, the Red Sea region, and eastern and central Asia. Our objectives were to quantify differences in aggressiveness among isolates representative of pre-2000 and post-2000 populations and to determine if increased aggressiveness of isolates likely contributed to the severity of these recent stripe rust epidemics. Seven isolates were evaluated at low (10 to 18C) and high (12 to 28C) temperature regimes for latent period, lesion length, lesion width, lesion area, and spore production on adult plants of a susceptible wheat cultivar. “New” isolates (since 2000) were significantly more aggressive than “old” isolates (before 2000) for all variables. At the low temperature regime, new isolates sporulated 2.1 days (16%) sooner, grew 0.3 mm per day (18%) faster, produced 999 (140%) more spores per inoculation site per day, and produced 6.5 (71%) more spores per mm^2 of lesion per day than old isolates. At the high temperature regime, new isolates sporulated 3 days (26%) sooner, grew 0.2 mm per day (18%) and 2.2 mm^2 per day (88%) faster, grew 1.2 mm (50%) wider, produced 774 (370%) more spores per inoculation site per day, and produced 6.2 (159%) more spores per mm^2 of lesion per day than old isolates. New isolates showed significant adaptation to the warm temperature regime for all variables. Based on these results and models for stripe rust epidemics, recent severe stripe rust epidemics can be at least partially explained by the pathogen’s increased aggressiveness, especially at higher temperature.

Intermediate infection types on differential lines cause discrepancies in race identification of *Puccinia striiformis* f. sp. *tritici* isolates

E. A. MILUS (2), S. G. Markell (1)
(1) North Dakota State University, Fargo, ND, USA; (2) University of Arkansas, Fayetteville, AR, USA
Phytopathology 98:S107

Puccinia striiformis f. sp. *tritici* causes stripe rust of wheat. In the U.S., races of this pathogen are identified based on infection types (IT) produced on a differential set comprised of 18 cultivars and two near isogenic lines. Infection types are rated on a 0 to 9 scale, with IT 0 to 4 considered avirulent and IT 5 to 9 considered virulent. Isolates from the eastern U.S. since 2000 have been given inconsistent race designations because the IT on four cultivars are intermediate (IT 4 to 6) and frequently fluctuate between avirulent (IT 4 and lower) and virulent (IT 5 and higher) reactions. Urediniospores of 16 isolates collected between 2000 and 2005 were produced simultaneously and used to inoculate two replicates of the differential set that were incubated in the same growth chamber that gradually changed from 8°C at midnight to 18°C at noon with a 14-hour photoperiod. IT recorded 14 days after inoculation produced conflicting race designations between the two replicate sets of differential lines for three isolates on Heines VII, four isolates on Produra, five isolates on Yamhill, and three isolates on Stephens. Infection types recorded 21 days after inoculation produced similar results. IT that fluctuate between virulent and avirulent reactions on these four cultivars can generate 16 race designations. Considering intermediate IT as “partially virulent” would be more accurate, but this is not compatible with the current system of designating races that requires a reaction to be either virulent or avirulent.

Microscopic surveillance of fluorescently tagged O157:H7 in spinach plants

R. MITRA (2), A. C. Wayadande (2), S. Lehman (2), P. Cuesta (1), S. E. Gilliland (1), J. Fletcher (2)
(1) FAPC/Dept. Animal Science, Oklahoma State University, Stillwater, OK, USA; (2) National Institute of Microbial Forensics, Food and Agriculture Biosecurity, Oklahoma State University, Stillwater, OK, USA
Phytopathology 98:S107

Recent outbreaks of food borne illness caused by the deadly enteric pathogen *Escherichia coli* O157:H7 on spinach have raised concerns about our ability to assure the safety of fresh foods for the consumer. The objective of this study was to confirm whether *E. coli* O157:H7 enters and colonizes growing spinach plants. Four week old spinach plants were inoculated with a mixture of five GFP-tagged *E. coli* O157:H7 strains using four different inoculation methods: leaf drop (spotting inoculum droplets on the abaxial leaf surface); pressure inoculation (bacterial inoculum forced into the leaf interior; root drench (introducing bacteria into the rhizosphere), and stab wounding (needle stabs into the stem). A total of 15 plants were inoculated with each treatment or with sterile PBS as a control. Plant samples taken at 1, 7, and 14 days after inoculation were examined by confocal laser scanning microscopy and the presence, location and density of GFP: *E. coli* O157:H7 was assessed. All experiments were repeated 4 times. No fluorescence was detected in any of the PBS controls. In plants inoculated by leaf drop, *E. coli* O157:H7 was detected on the leaf surfaces of all 9 plants as long as 2 weeks post inoculation. Fluorescing E. coli O157:H7 was detected for up to 7 days in 5 of 9 plants in which the pressure inoculation method introduced bacteria into the intercellular spaces of the mesophyll. After root drenching, the pathogen was found associated with soil particles or on the surfaces of root hairs in 3 of 9 plants. Stab wound inoculation resulted in detection of fluorescence in 1 of the 9 treated plants. The results suggest that under some of our treatment conditions *E. coli* O157:H7 can colonize on spinach plant surfaces. Whether such colonization can, or is likely to, occur in nature remains unknown.

Ammonia secretion and ambient pH as virulence regulators of *Colletotrichum gloeosporioides* pathogenic on avocado fruits

I. MIYARA (2), H. Shafran (1), A. Sherman (1), D. Prusky (2)
(1) Department of Genomics, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel; (2) Department of Postharvest Science, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel
Phytopathology 98:S107

Accumulation of ammonia and associated tissue alkalinization, predispose avocado fruit to attack by *Colletotrichum gloeosporioides*. A macroarray carrying 10,000 unsequenced EST was constructed from *C. gloeosporioides* in order to determine the different genes involved in the alkalinization process and fungal pathogenicity. A group of nitrogen related genes, including: glutamine synthase (*GS*), nad^+ specific glutamate dehydrogenase (*GDH2*), ammonia transporters (*MEPA*, *MEPB*) and glutamate transporter (*GLT*), were monitored, and their roles in ammonia release were determined. Furthermore it was found that gene knockout of *GDH2* encoding for glutamate dehydrogenase resulted in: the reduction of ammonia production; the loss of the ability to alkalinize the ambient pH; reduction in the production of pectate lyase (PL) as well as reduction of *C. gloeosporioides* pathogenicity. For this reason we studied the regulation of PL production by manipulation of *C. gloeosporioides* pH transcription factor PacC. It was found that loss-of-function *PAC1* mutants showed high reduction of *PELB* transcript expression, delayed PL secretion and dramatically reduced virulence, as detected in infection assays with avocado fruits. The above results suggest that *C. gloeosporioides* activate alkaline pH by ammonia accumulation and this alkaline pH is essential for activation of its pathogenicity.

Disease resistance in commercial cultivars of *Hydrangea macrophylla*

M. T. MMBAGA (1), S. M. Reed (2), M. Windham (4), L. Yonghao (4), T. A. Rinehart (3)
(1) Tennessee State University; (2) USDA-ARS, McMinnville; (3) USDA-ARS, Poplarville; (4) University of Tennessee, Knoxville
Phytopathology 98:S108

Hydrangea macrophylla is a popular summer-flowering shrub, grown primarily for its large, brightly colored inflorescences. Although powdery mildew has been well-documented in this species, germplasm has not been evaluated for resistance to this and other diseases. Ninety *Hydrangea macrophylla* cultivars were evaluated for resistance to foliar diseases in McMinnville, TN in 2006 and 2007. The experiment was a completely randomized design with three replications. Powdery mildew was the main disease observed in both years. Although most cultivars were susceptible to powdery mildew, eight cultivars (*H. macrophylla* ssp. *macrophylla* 'Tricolor', 'Lemon Wave' and 'Veitchii' and *H. macrophylla* ssp. *serrata* 'Hokaido', 'Pretty Maiden', 'Miyama Yae Murasaki', 'Omacha' and 'Shirofuji') exhibited the highest resistance to powdery mildew in both years. While *H. macrophylla* ssp. *macrophylla* 'Komachi' and 'Endless Summer' and *H. macrophylla* ssp. *serrata* 'Blue Billow', 'Shirofuji' and 'Hallasan' exhibited powdery mildew resistance in 2006, these cultivars had only moderate resistance in 2007. Approximately 4% of the 69 *H. macrophylla* ssp. *macrophylla* and 44% of the 18 *H. macrophylla* ssp. *serrata* cultivars evaluated were resistant and all three hybrids between the two subspecies were susceptible. In 2007, many cultivars developed leaf blights, leaf spots, flower blight, and dieback and plants defoliated prematurely. Fungi isolated from necrotic lesions included *Alternaria*, *Cercospora*, *Ascochyta*, *Colletotrichum*, and *Septoria* species. Pathogenicity tests confirmed that *Alternaria* and *Ascochyta* were primary pathogens on 'Blue Nikko', 'Blue Bird' and 'All Summer Beauty' causing leaf blight symptoms. Although *Cercospora*, *Colletotrichum*, and *Septoria* are pathogens of hydrangea, their pathogenicity was not confirmed, perhaps due to the cultivars or the temperature conditions used in pathogenicity tests. These results suggested that leaf blight diseases observed in hydrangea foliage is a disease complex involving several pathogens. The results also suggested that more effort is needed to incorporate powdery mildew resistance in *H. macrophylla*.

Fungal trunk pathogens associated with grapevine decline in Iran

H. Mohammadi (2), J. Armengol (1), Z. BANIHASHEMI (2)
(1) Instituto Agroforestal Mediterraneo, Universidad Politecnica de Valencia, Camino de Vera s/n, 46022- Valencia, Spain; (2) Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran
Phytopathology 98:S108

Grape (*Vitis vinifera* L.) is an important fruit crop in Iran and covers an area of 250,000 ha with 21,250,000 tons annual production. A field survey from vineyards in different areas of Iran was conducted from 2004 to 2007 in order to determine the fungal pathogens associated with vine decline. From each visited vineyard, samples were taken from grapevine showing yellowing and necrosis of leaves, reduced growth, and wood necrosis and xylem discoloration in cross section. Isolations were made from affected wood tissues from branches, trunks and crowns onto MEAS. All of the isolates were identified based on morphological and molecular characteristics. *Phaeoacremonium* species were identified using a PCR-RFLP technique with the specific primers pm1 and pm2 and the restriction enzymes *Bss*KI, *Eco*O1091 and *Hha*I. The species identification were also confirmed by sequencing the beta-tubulin gene using primers T1 and Bt2b. *Phaeomoniella chlamydospora* and *Cylindrocarpon* sp. isolates were identified based on beta-tubulin gene sequences and *Botryosphaeria* sp. isolates were identified by sequencing the elongation factor 1-alpha (EF1) gene. The fungi that were found associated with grapevine decline in Iran were: *Phaeoacremonium aleophilum*, *Phaeoacremonium parasiticum*, *Phaeomoniella chlamydospora*, *Diplodia seriata*, *Neofusicoccum parvum*, *Cylindrocarpon liriodendr Phoma* sp., *Phialophora* like fungi and *Acremonium* sp.

Characterization and differentiation of Erwinia amylovora strains from Iran

M. MOHAMMADI (2), K. Geider (1)
(1) Julius Kuhn Institute (JKI), Shwabenheimer Str. 101 D-69221 Dossenheim, Germany; (2) University of Tehran, Dept. of Plant Pathology, Karaj, Iran
Phytopathology 98:S108

A total of 27 strains of Erwinia amylovora (Ea) representing Iran, Spain and the Netherlands were characterized and compared according to their phenotypical as well as molecular properties. All Ea strains produced levan on LB sucrose plate to a varying degree, formed yellow colonies on MM2Cu selective medium, showed no growth on MM1Cu plate, produced slime on MM2C plate and induced the hypersensitive reaction (HR) on tobacco leaves. Amylovoran synthesis in MM2C medium, siderophore excretion, level of tolerance to oxidative stress and degree of oozing and tissue necrosis on immature pear slices were highly variable. Chemotaxis experiments revealed a wide variation in response to utilization of several amino acids and organic acids. All Ea strains amplified a 1.0 kb PstI fragment present on the ubiquitous pEA29 plasmid with the exception of one strain. E. amylovora strains were further identified using a new and specific primer pair set, 119/Y3 and 119/X12, which amplified a 0.6 kb amsJ chromosomal fragment involved in the synthesis of exopolysaccharide. Ea strains were differentiated based on their short sequence DNA repeat (SSR) pattern of plasmid pEA29. SSR number among the strains ranged from four to nine. The nucleotide sequence of the house keeping ompA gene was identical among Ea strains from fruit trees but slightly different from those of Rubus and other strains. Pulse-field gel electrophoresis (PFGE) showed no differences in XbaI-restricted DNA pattern among the representative strains from Iran and thus they were all classified as type 2 (Pt2), typical for the Mediterranean region. It is possible that the fire blight pathogen entered Iran through the borders with Turkey in the northwest.

The Hsp90 inhibitor, geldanamycin, down-regulates genes involved in thermotolerance and pathogenicity in *Magnaporthe oryzae*, the rice blast fungus

M. Mohammadi (1), K. HUANG (1), N. M. Donofrio (1)
(1) University of Delaware
Phytopathology 98:S108

Geldanamycin (GDA) is an antibiotic produced by the soil-inhabiting bacterium, Streptomyces hygroscopicus var. geldanus and used, along with its analogs, as an anti-cancer therapeutic drug to suppress proliferating malignant cells. GDA inhibits the heat shock protein Hsp90, a highly conserved molecular chaperone in eukaryotic cells, by specifically binding to the middle domain of Hsp90 where cochaperones normally bind. Hsp90 is involved in the activation and maturation of a large number of client proteins during normal cell growth and development as well as under stress conditions by forming a multichaperone complex with its cofactors and cochaperones. We used GDA to explore the role of Hsp90 in pathogenicity of the rice blast fungus, *Magnaporthe oryzae*, and found several developmental and virulence-related stages to be inhibited, including mycelial growth, spore germination, appressorium formation and host symptom development. Microarray analysis revealed down-regulation of 500 genes in GDA-treated fungal mycelium. Included in this gene set is FKBP504, a heat-inducible, immunophilin cochaperone of Hsp90. Deletion mutants of the FKBP504 gene were significantly less thermotolerant at 32°C and did not induce blast lesion formation on rice and barley leaves inoculated with heat-shocked spores, in contrast to wild type. Similar results were obtained with two other Hsp90 cochaperones, Aha1 and Sba1. We conclude that Hsp90 cochaperones seem to be required for symptom expression on host leaves following a heat shock treatment of fungal spores.

Tropical Race 4 of *Fusarium oxysporum* f. sp. *cubense* causing new Panama wilt epidemics in Cavendish varieties in the Philippines

A. B. MOLINA (1), E. G. Fabregar (4), V. Sinohin (1), G. Fourie (3), A. Viljoen (2)
(1) Bioversity International-Philippines, Los Banos, Philippines; (2) Department of Plant Pathology, University of Stellenbosch, South Africa; (3) FABI, University of Pretoria, South Africa; (4) Lapanday Foods Corporation, Davao Philippines
Phytopathology 98:S108

Tropical Race 4 (TR4), the virulent strain of *Fusarium oxysporum* f. sp. *cubense*, is a serious threat to the export Cavendish-based banana industry. TR4 has not been reported in the Philippines until recently when Panama wilt infections were observed in several commercial Cavendish farms. To confirm the identity of the new epidemics, a survey was conducted from September to December 2005. Infected plants showing the typical Panama wilt symptoms were collected from several commercial farms. Samples were sent to the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa for Vegetative Compatibility Group (VCG) analyses. Results confirmed that the pathogens isolated from infected samples were identified to belong to VCG 1213/16 complex, the VCG known to be associated with TR4. To quantify the severity of epidemics, the incidence of Panama wilt cases were monitored in two farms. The incidence of Panama wilt increased from 700 cases in 2005 to 15,000 cases in 2007. The new TR4 epidemic threatens the long term sustainability of the Philippine banana industry and its dominance in the banana exports in Asia. The spread of TR4 is also a threat as a transboundary pathogen to other Cavendish producing countries and continents.

Population dynamics and spatial distribution of *Rotylenchulus reniformis* upon introduction into a cotton field

S. R. MOORE (1), K. S. Lawrence (1), F. J. Arriaga (2), E. van Santen (1), C. H. Burmester (1)
(1) Auburn University, Auburn, AL, USA; (2) USDA-ARS, Auburn, AL, USA
Phytopathology 98:S109

Rotylenchulus reniformis has become the most important nematode pathogen of cotton in Alabama. The knowledge of how population levels grow upon introduction into a cotton field could prove to aid in control measures. Vermiform life stages of *R. reniformis* were injected into selected rows of a previously non-infested cotton field at planting and movement was monitored. Two fields, one irrigated and the other non-irrigated were inoculated and replicated 10 times. Horizontal movement was monitored by taking soil samples from the top 15 cm of soil every thirty days. Data were statistically analyzed using GLM, and means compared using Fisher's protected least significant difference test. *Rotylenchulus reniformis* was observed to move an average of 75 cm away from the inoculated row throughout the season over all replications in both the irrigated and non-irrigated trials. Maximum distance moved was 2 m from the inoculated row. Populations in the irrigated trial were highest ($P < 0.05$) in the inoculated row at 60 and 120 days after planting (DAP), and higher ($P < 0.05$) at 30 and 90 DAP within 50 cm of the inoculated row. Populations were similar within 2 m of the inoculated row at harvest. Populations in the non-irrigated trial were higher ($P < 0.05$) in the inoculated rows for all sampling dates except for the 120 DAP sample where no differences could be determined within 1 m of the inoculated row. Populations in both the irrigated and non-irrigated trials followed a negative parabola with the highest peak at the inoculated row. Maximum populations within the inoculated rows were observed to reach 881 and 433 vermiform life stages/150 cm3 in the irrigated and non-irrigated trials respectively. At a distance of 1 m from the inoculated row, populations were 201 and 216 per 150 cm^3. These results illustrate the speed at which *R. reniformis* can spread within a cotton field.

Evidence for geographic isolation and distinct patterns of recombination in the aflatoxin gene cluster of *Aspergillus flavus*

G. G. MOORE (3), B. W. Horn (6), J. L. Elliott (3), K. Hell (5), S. N. Chulze (1), G. Wright (4), M. K. Naik (2), I. Carbone (3)
(1) Departamento de Microbiologia e Inmunologia, Universidad Nacional de Rio Cuarto, Cordoba, Argentina; (2) Department of Plant Pathology, College of Agriculture, Karnataka, India; (3) Department of Plant Pathology, North Carolina State University, Raleigh, NC 27606; (4) Department of Primary Industries, Queensland, Kingaroy, Australia; (5) International Institute of Tropical Agriculture, Cotonou, Republic of Benin; (6) National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, GA 39842
Phytopathology 98:S109

Aflatoxins are toxic compounds produced by several *Aspergillus* species that contaminate food crops worldwide. *A. flavus* is the most common agent of aflatoxin contamination of corn, peanuts, cottonseed, figs and tree nuts in the U.S. Extensive studies have elucidated the biochemical and regulatory mechanisms of aflatoxin production, but basic knowledge of the evolutionary processes that maintain toxicity in *A. flavus* is lacking. We sequenced 21 intergenic regions in the aflatoxin gene cluster for a sample of 43 L-strain isolates of *A. flavus* representing the genetic diversity within a single peanut field in Georgia. Linkage disequilibrium analyses revealed six distinct recombination blocks that separate seven contiguous genes in the cluster (*aflE*, *aflM*, *aflN*, *aflG*, *aflL*, *aflI*, and *aflO*). This block-like organization is the result of recombination among haplotypes representing distinct vegetative compatibility groups. Subsequent screening of molecular sequence variation in these blocks in populations of *A. flavus* L-strain from Africa, Argentina, Australia, and India revealed shared haplotypes with the Georgia population as well as geographic-specific haplotypes. Although recombination blocks were not detected in the Australian and Indian populations, a similar block-like organization was found in other populations. We also detected significant geographic differentiation for two noncluster genes. Whether this isolation is consistent with restricted gene flow, or indicative of other demographic/ ecological processes, is currently under investigation.

New host associations and potential overwintering strategies of *Melampsora* species on poplar and willow in North America

L. L. MOUNT (1), J. A. Smith (1), R. A. Blanchette (2)
(1) University of Florida, Gainesville, FL, USA; (2) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S109

In North America, the taxonomy of rust fungi that parasitize the Salicaceae does not always reflect biology or pathogen phylogeny. Disease resistance evaluations of poplar (*Populus* spp.) using field plots, and surveys of cultivated and natural populations of willow (*Salix* spp.) species, were used to identify new hosts and potentially new rust species. Collections were made between 2004 and 2008 in Nunavut, Canada; Minnesota and Florida, U.S.A. For all isolates, internal transcribed spacer region rDNA (ITS-rDNA) sequences were obtained and compared against those in GenBank. Based on phylogenetic analysis of ITS sequences, isolates from hosts of *Salix* section Chamaetia in Arctic Canada exhibit marked host-specific clustering, suggesting speciation within the *M. epitea* complex. In addition, we report several new species of poplar and willow as hosts of *Melampsora* including: *P. charbinensis*, *P. hsinganica*, *S. re*pens var. argentea in Minnesota and *P. deltoides* × *yunnanensis* 'Kawa' and *P. yunnanensis* in Florida. Of particular interest is the first report in North America of weeping willow (*S. babylonica*) and ramshorn willow (*S. babylonica* cv. 'Crispa') as hosts for *M. epitea* sensu lato. In Florida, weeping willow retains partial foliage until new leaves emerge in spring. This apparently serves as an overwintering strategy and reservoir for the fungus and aids in disease development in the southern United States. Also, another cultivar of *S. babylonica*, 'Yatsubusa' was not infected by *Melampsora* spp. despite nearby infections of other clonal cultivars of the same species. The identification of resistant cultivars may become significant for continued use of this willow species in the region.

Assessment of new inoculation methodologies to evaluate Biocontrol agents on Fusarium crown rot of wheat

E. A. MOYA (1), B. J. Jacobsen (1)
(1) Montana State University, Bozeman, MT, USA
Phytopathology 98:S109

Fusarium Crown Rot (FCR) of wheat is a persistent problem that causes significant losses in the Northern Great Plains and other semiarid regions of the world. Biological control agents (BCAs) have shown promise for the control of the plant diseases, however adequate bioassay methods used to assess the performance of BCAs on FCR have not been identified. We have assessed bioassays using different layers of inoculated soil relative to seed placement in pots (upper inoculation, lower inoculation and layer below seeds), using drop inoculation by applying 1 mL of a suspension with macroconidia of *Fusarium culmorum* with the seed, and using a perforated microcentrifuge tube inserted in the center of the pot soil and equidistant of the emerged plants. These methodologies were assessed with three different rhizobacteria, *Trichoderma harzianum*, and the seed treatment fungicide difenoconazole (Divident, Syngenta) using the spring red cultivar Hank. The methods of inoculation showed differences ($P \leq 0.05$) among them when FCR severity was assessed. A layer of infested soil located below the seed gave the lowest disease severity of the methods used. Infested soil layers placed above the seed provided the highest disease severity followed by inoculation with the seed. *F. culmorum* inoculated directly on roots using the micro-centrifuge tube provided homogeneous infestation and high disease severity when concentrations over 5,000 macroconidia per gram of soil were applied. Using this method, a linear relationship between disease severity and macroconidia concentration was identified. The primary advantage of the microcentrifuge tube inoculation method is avoiding bias associated to non-germinated seeds and uniformly placing the inoculum in the root and coleoptile infection courts. Only difenoconazole seed treatment significantly ($P < 0.05$) reduced disease severity.

Seasonal release of ascospores by *Erysiphe necator*

M. M. MOYER (1), D. M. Gadoury (1), W. F. Wilcox (1), R. C. Seem (1)
(1) Dept. of Plant Pathology & Plant-Microbe Biology, NYSAES, Cornell University, Geneva, NY, USA
Phytopathology 98:S109

Cleistothecia are an important source of primary inoculum for grapevine powdery mildew (*Erisyphe necator*), and the only source of primary inoculum in viticulture areas with relatively cold winters, such as NY (USA). Previous studies indicated that ascospores were released from budbreak to shortly after bloom in NY during rain events of 2.5 mm or more, coincident with temperatures above 10°C. To better understand how ascospore release is affected by overwintering conditions, we collected populations of cleistothecia from NY, WA, NC, NJ, GA, VA, and PA (USA), overwintered the same in NY, and also overwintered NY cleistothecia in WA, NC, NJ, GA, VA, and PA during 2006–7 and 2007–8. When subjected to conditions conducive for dehiscence, cleistothecia from all sites released ascospores starting in midwinter. In several site*year combinations, ascospore release was nearly complete before local budbreak of grapevines. Once initiated, the ascospore supply was often depleted within 100 DD (Base = 10°C). Severity of powdery mildew varies greatly from year to year in many viticultural regions, and this may be partially due to variation in the quantity of inoculum available at budbreak. Our results suggest that ascosporic inoculum can be reduced if multiple favorable rain events occur before budbreak.

Sensitivity of ELISA and RT-PCR in detection of *Tomato ringspot virus* in apple cultivars

W. MSIKITA (1), T. Kell (1), R. Welliver (1)
(1) Pennsylvania Department of Agriculture, Harrisburg, PA 17110
Phytopathology 98:S110

Accurate disease detection impacts important programs such as breeding for disease resistance, certification, and disease control. In earlier study we noted variability between the enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) in detecting *Tomato ringspot virus* (ToRSV) in a commercial apple orchard in Pennsylvania. In the present study three commercially available ELISA kits were tested for their ability to detect ToRSV in 90 samples collected from dead and declining apple cultivars: 'Cameo', 'Golden glory', 'Honeycrisp', and 'York'. Samples were processed and tested for ToRSV by the double-antibody enzyme-linked immunosorbent assay (DAS-ELISA) following manufacturers' recommendation. Of the three kits tested, two did not detect ToRSV in any of the samples, whereas one kit detected the virus in 2.5% of the samples, and all from the cultivar 'Golden glory'. Using RT-PCR however, ToRSV was detected in all 4 cultivars such that detection frequency varied significantly among cultivars, and ranging from 33% in cultivar 'York' to 85% in 'Golden glory'. The study examines the cultivars in relation to differences in ToRSV detection both by immuno- and nucleic acids-based detection techniques.

Functional characterization of heterotrimeric G protein regulators in *Fusarium verticillioides*

M. MUKHERJEE (1), W. Shim (1)
(1) Dept. Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77843-2132, USA
Phytopathology 98:S110

Fumonisin B_1 (FB_1), a mycotoxin produced by the fungus *Fusarium verticillioides*, pose considerable food safety and economic concerns. Previously, *GBB1*, a heterotrimeric G-protein beta subunit gene, was shown to be a key regulator of FB_1 biosynthesis. In this study, we performed functional analyses of genes that encode putative RGS (regulators of G protein signaling) and PhLP (phosducin-like protein) proteins in *F. verticillioides*. These proteins are known to regulate heterotrimeric G-protein activity by altering the intrinsic GTPase activity or by direct binding, which in turn impacts signaling mechanisms that control fungal growth, virulence and secondary metabolism. Our aim was to isolate and characterize gene(s) downstream of *GBB1* that is directly associated with FB_1 regulation. We identified eight genes (2 PhLPs and six RGSs) in the *F. verticillioides* genome. Transcriptional expression study revealed that three RGS genes were up-regulated in the *gbb1* deletion mutant and one RGS gene was up-regulated in the wild type. To characterize their role in FB_1 biosynthesis, we generated knock-out mutants by homologous recombination strategy. PCR and Southern analyses confirmed the knock-out mutations in all four targeted RGS genes. Phenotypic characterization of four mutants, particularly FB_1 production, will be presented. Genetic complementation will also be performed to verify the mutation.

New tombusviruses isolated from waters draining forest stands in New Zealand

S. S. MUKHERJEE (3), T. J. Lough (1), D. H. Hopcroft (2), M. R. Woodford (3), J. D. Castello (3)
(1) Genesis Research and Development Corporation Ltd., Auckland, New Zealand; (2) Massey University, Palmerston North, New Zealand; (3) SUNY College of Environmental Science and Forestry, Syracuse, NY 13210
Phytopathology 98:S110

Systemic but asymptomatic plant viruses have been previously proposed as ideal gene vector candidates for genetic improvement of forest trees. Water samples were collected from Turitea Creek and the Manawatu River near Palmerston North, New Zealand to assay for systemic but asymptomatic plant viruses that infect eucalyptus and radiata pine in nearby forest stands. Twenty liter water samples were prefiltered and virions adsorbed to electropositive Zeta Plus 50S membranes. The eluates were examined for virions by transmission electron microscopy. Icosahedral particles with a modal diameter of 30 nm with no distinct capsomere arrangement were observed. Two distinct viruses were isolated based on symptomatology in *Vigna unguiculata.* An $A_{260/280}$ ratio of 1.64 and a buoyant density of 1.35 in CsCl supported the conclusion that both isolates were tombusviruses. Maximum Parsimony trees generated from an 820 bp amplicon within the replication-associated p33 gene showed maximum similarity of the Turitea Creek Virus with the TBSV-Japanese isolate (81%) and sequencing the entire 1100 bp capsid protein gene produced maximum similarity with AMCV (73%). Nucleotide sequences of the Manawatu River isolate in the p33 and capsid protein regions produced similarities of 81% and 89%, respectively, with the cherry strain of Tomato Bushy Stunt Virus. Distinctive host range and symptomatology, especially in hosts in the family Cucurbitaceae, combined with sequence differences with well-described tombusviruses suggest that both isolates are new members of the tombusvirus genus. The presence of these infectious tombusviruses in waters draining forest stands in New Zealand suggests their potential as gene vectors in future tree improvement programs, if they are determined to infect eucalyptus or radiata pines. To our knowledge, this is the first report of tombusviruses in New Zealand.

A geographic information systems (GIS) analysis of soybean rust distribution at the field level

P. E. MUMMA (1), R. W. Schneider (1), L. Wang (1)
(1) Louisiana State University, Baton Rouge, LA, USA
Phytopathology 98:S110

Since the discovery of soybean rust in the continental United States in 2004, there has been considerable interest in epidemiological details related to long distance and local spread of the disease. In this study, a geographical information systems (GIS) analysis was used to investigate within-field disease distribution. Research was focused on determining the extent of spatial and temporal variability of soybean rust with regard to soil nutrients, soil compaction, leaf nutrients, percent canopy coverage, soil moisture, and plant height and their interactions. The study areas included three soybean fields with a total area of about 15 acres. The fields were located close to Baton Rouge, LA. Using a grid pattern, each field was divided into 50 sample sites, and GPS coordinates were recorded at each site. The sites were rated twice during the 2007 growing season to quantitatively assess disease severity. ArcMap was used to perform exploratory data analysis, including correlations among variables, variogram analysis of the spatial structure of each variable, and surface interpolation, with the results being displayed graphically. These results will be presented and discussed.

Longevity of inoculum production by *Diplodia pinea* on red pine cones

I. A. MUNCK (1), G. R. Stanosz (1)
(1) Department of Plant Pathology, University of Wisconsin-Madison, WI, USA
Phytopathology 98:S110

Diplodia pinea causes shoot blight, stem cankers, and death of the native red pine (*Pinus resinosa*) in northcentral and northeastern North America. This fungal pathogen sporulates abundantly on mature, open seed cones. In summer of 2004, pycnidia of *D. pinea* were observed on cones that had matured and opened during previous years, but had been retained in canopies of trees at a mature red pine plantation in southern Wisconsin. This observation prompted surveys during the winter and early summer of three consecutive years (2005–2007) to determine incidence and abundance of *D. pinea* conidia on cones of different ages in this stand. During each collection time, cones from three different age classes were collected from the canopy of each of ten trees. Cones from each age class consistently bore pycnidia with conidia of *D. pinea*. A water washing and filtration technique was used to quantify *D. pinea* conidia extracted from colonized cones. Although cones collected in June of the year after their maturation tended to yield more *D. pinea* than older cones, large numbers of conidia were obtained from cones even 3 years after they had matured. Perennial availability of inoculum due to persistence of *D. pinea* on cones of several ages in the overstory or in adjacent stands should be considered when regenerating red pine in areas where this pathogen is known to be present.

Watermelon mosaic virus incidence and yield losses in summer squash reduced by use of UV-reflective plastic mulch but not biological control

J. F. MURPHY (1), J. Masiri (1), M. Eubanks (2)
(1) Auburn University, AL, USA; (2) Texas A&M University, College Station, TX
Phytopathology 98:S110

We evaluated the integration of UV-reflective plastic mulch and a commercial biological control treatment, BioYield™, to reduce the incidence and yield losses in Summer squash caused by the aphid-borne plant virus, *Watermelon mosaic virus* (WMV). In the spring trial, there was significantly reduced WMV incidence and whole treatment average ELISA values among squash plants grown on silver-on-black mulch compared with plants grown on non-reflective black mulch. Significantly greater squash fruit yields were obtained for plants grown on the silver-on-black mulch relative to the black mulch treatment. In the fall trial, highly UV-reflective silver mulch was used in addition to silver-on-black and black mulches. WMV incidence and whole treatment average ELISA values were significantly lower for squash plants in the silver mulch treatment compared with silver-on-black and black mulch treatments. The silver-on-black treatment resulted in lower WMV incidence and whole treatment average ELISA values compared with the black mulch treatment. Squash plant yields were significantly greater for plants in the silver-on-black mulch treatment than for those in the silver or black mulch treatments. Treatment of squash plants with BioYield™ did not reduce virus

incidence or whole treatment average ELISA values nor did it result in higher squash fruit yields compared with the non-treated control in spring or fall trials.

Identification of plant defense signaling components induced in response to fungal elicitor EIX

S. MUTHAPPA (2), C. Ryu (2), M. Sharfman (1), A. Avni (1), K. S. Mysore (2)
(1) Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel; (2) Noble Foundation, Ardmore, OK, USA
Phytopathology 98:S111

Plant microbe interactions involve a large number of global regulatory systems, which are essential for plants to protect themselves against pathogen attack. An ethylene-inducing xylanase of Trichoderma viride (tvEIX) is a potent elicitor of plant defense responses, like hypersensitive response (HR) in specific cultivars of tobacco and tomato. The molecular genetic analysis of the signal transduction pathway that modulates HR is an important step in understanding the plant defense responses. In this study, we attempted to identify signaling components involved in tvEIX and its cognate *R* gene of tomato, *LeEix2*, in inducing HR in *Nicotiana benthamiana*. We used virus-induced gene silencing (VIGS)-based fast-forward genetics approach to screen about 3,300 cDNA clones from a mixed elicitor treated and normalized *N. benthamiana* cDNA library and documented the responses of these plants to co-expression of tvEIX and LeEix2. *Agrobacterium*-mediated transient co-expression of LeEix2 and tvEIX in the leaves of *N. benthamiana* plants showed HR whereas several gene-silenced plants showed differential HR symptoms like delayed HR, early HR, and no HR. To identify EIX specific responses, we simultaneously observed HR mediated by two other gene-for-gene interactions Pto-avrPto and Cf9-avr9 as positive controls. Based on this screening approach we identified about 10 candidate genes that when silenced clearly compromised LeEix2-tvEIX mediated HR. In order to achieve uniform HR which might facilitate better screening and characterization of identified signaling genes, we also developed *N. benthamiana* transgenic plants stably expressing *LeEix2* gene.

Identification of genes involved in nonhost disease resistance in *Nicotiana benthamiana* and *Arabidopsis thaliana*

S. MUTHAPPA (1), K. Wang (1), C. Ryu (1), K. S. Mysore (1)
(1) Noble Foundation, Ardmore, OK, USA
Phytopathology 98:S111

Plants have evolved different defense mechanisms, both active and passive, to combat pathogen attack. The well studied *R*-gene mediated defense is often very specific to a particular plant genotype or cultivar and a particular race of a pathogen. In contrast, nonhost resistance can act against all races of a particular pathogen and can occur in all cultivars of a host plant species. In this study we attempted to dissect the signaling pathway during nonhost pathogen and plant interaction. We used virus-induced gene silencing (VIGS) based fast-forward genetics approach and screened a cDNA library to identify the candidate genes possibly involved in imparting nonhost resistance in *N. benthamiana*. GFPuv labeled nonhost pathogenic bacterial growth patterns was studied in these silenced plants. We documented the differences in nonhost bacterial growth between the mock and the gene silenced plants. We have identified several candidate genes that when silenced compromises nonhost resistance in *N. benthamiana*. We are now characterizing the function of these genes and their relevance to plant defense using gene overexpression, RNAi and Arabidopsis mutants.

Differences in constitutive and induced expression of two phenolic compounds in coast live oaks susceptible and resistant to infection by *Phytophthora ramorum*

A. M. NAGLE (2), M. Garbelotto (1), P. Bonello (2)
(1) Dept. of Environmental Science, Policy, & Management, University of California, Berkeley, CA 94720, USA; (2) Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210, USA
Phytopathology 98:S111

Phytophtora ramorum is the causal agent of sudden oak death (SOD), a devastating disease of tanoaks and oaks in California and Oregon. Apparent resistance to infection by *P. ramorum* in coast live oak (CLO) has been observed in natural populations and in laboratory inoculation trials. No practical controls for this disease are available, therefore characterization of natural resistance is highly desirable. In a preliminary test, we used HPLC analysis to evaluate branch phloem phenolic profiles for CLO's previously identified as relatively susceptible (S) (N = 4) or resistant (R) (N = 5) to *P. ramorum*. Separate sets of branches from the same trees were wound-inoculated with *P. ramorum*. Two compounds, identified as tyrosol and catechin, were present in constitutively higher amounts in R than in S trees, but due to the low replication the differences were not significant. However, a significant overall negative correlation was found between lesion length and tyrosol concentration ($r = -0.667$, $N = 9$, $P = 0.026$). These preliminary findings may be important in establishment of chemical biomarkers, which has great significance in applications such as screening of oak germplasm for resistance to SOD. Follow-up studies in different seasons and with trees exhibiting prolonged resistance in the field under high disease pressure are planned.

Development of real-time PCR for the detection of exotic potyviruses infecting imported plant germplasm

M. K. NAKHLA (1), K. J. Owens (1), W. Li (1), L. Levy (1)
(1) USDA APHIS PPQ CPHST, Beltsville, MD 20705, USA
Phytopathology 98:S111

Several viruses can infect and move with imported germplasm causing serious threats to American agriculture. We developed fast, sensitive and reliable real-time PCR primers and probes for the detection of four grass-infecting viruses and six sweet potato-infecting viruses. These assays are an improvement over the conventional PCR assays for grass and sweet potato-infecting potyviruses that we developed last year. Sequences of grass-infecting potyviruses [*Cocksfoot streak virus* (CSV), *Johnsongrass mosaic virus* (JGMV), *Maize dwarf mosaic virus* (MDMV), and *Sugarcane mosaic virus* (SCMV)] and sweet potato-infecting viruses [*Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus G* (SPVG), *Sweet potato virus Y* (SPVY), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), and *Sweet potato mild speckling virus* (SPMSV)] were used to design TaqMan probes and primers. Partial viral sequences (viral mini-genes) were synthesized and used to evaluate, optimize and standardize quantitative real-time PCR (qPCR) assays. All the qPCR assays developed showed a high sensitivity, with detection levels as low as 30 copies of the synthetic targets. Our results demonstrated a viable approach of using synthetic target DNA (mini-genes) of restricted and unavailable pathogens for the development of reliable diagnostic tools. The real-time PCR primers and probes developed in this study promise to be useful tools for screening foreign plant germplasm.

Development of real-time PCR for the detection and identification of potato cyst nematode

M. K. NAKHLA (1), K. J. Owens (1), W. Li (1), L. Carta (3), A. Skantar (2), L. Levy (1)
(1) USDA APHIS PPQ CPHST, Beltsville, MD 20705, USA; (2) USDA ARS Molecular Plant Pathology Laboratory, Beltsville, MD 20705, USA; (3) USDA ARS Nematology Laboratory, Beltsville, MD 20705, USA
Phytopathology 98:S111

Potato cyst nematode (PCN), *Globodera pallida*, a major potato pest and a quarantine pest in the USA, was detected in Idaho for the first time in 2006. PCN detection and identification is based on morphological features followed by conventional PCR based protocols. In this study, we developed TaqMan primers and probes for PCN detection and identification using real-time PCR. The primer set (PITS PAL3mf/PITSp4) with the TaqMan probe Gp were used to successfully detect and identify *G. pallida*. Another TaqMan probe (Prosto-rtP) was used for the detection of *G. rostochiensis* and *G. tabacum*. Prosto-rtP probe was also used with the primer set (Prosto-rt1f/Prostartr) for the identification of *G. rostochiensis* and used with the primer set (Prosto-r2f/PITST3) for the identification of *G. tabacum*. Multiplexing the real-time PCR assays for the detection and identification of two species at a time were also successful.

Molecular characterization of the PhoP/PhoQ two-component signal transduction system in *Erwinia amylovora*

S. NAKKA (1), Y. Zhao (1)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL 61801
Phytopathology 98:S111

The PhoP/PhoQ system is a pleiotropic two-component signal transduction system that controls many pathogenic properties in several animal and plant pathogens, and is a master regulator of virulence genes in *Salmonella*. Three different cues have been proposed to activate the PhoP/PhoQ system: a mild acidic pH; antimicrobial peptides and low Mg^2. The role of the PhoP/PhoQ system in *Erwinia amylovora* pathogenesis is unknown so far. In this study, phoP/phoQ deletion mutants of *E. amylovora* were generated and characterized. Our results showed that, while phoP, phoQ and phoPQ double mutants were still pathogenic on immature pear fruit, these mutants were more resistant to strong acidic conditions than the wild type strain (WT). The growth of the mutants was same as WT at pH 5.5 and 7 in modified basal medium A (MBMA) at low Mg^2 concentration; however, the growth of the mutants at pH 4.5 and 5 was greatly increased. At 24 h, the bacterial number of the mutants was ~100 fold higher than that of the WT at pH 4.5. At pH 5.5 and low Mg^2 concentration, we also found that the survival of the mutants was ~ 2 and 10 times less than that of the WT when treated with antimicrobial peptides, thionin and cecropin A, respectively, suggesting that the PhoP/PhoQ system renders the pathogen more resistant to antimicrobial peptides. Further analysis demonstrated that phoP/phoQ mutants were more sensitive to osmotic stress and iron than the WT strain at acidic pH as well. In addition,

the phoP/phoQ mutants showed irregular and reduced swarming motility on swarming plates containing 0.3% agar. These results indicate that the PhoP/PhoQ system, although does not function in virulence, plays an important role in the survival of the pathogen in stress conditions.

Analysis of gene expression in Jupiter rice showing partial resistance to rice panicle blight caused by *Burkholderia glumae*
R. NANDAKUMAR (1), M. C. Rush (1)
(1) LSU-Agcenter, Baton Rouge, LA, USA
Phytopathology 98:S112

The rice bacterial panicle blight (BPB) pathogen *Burkholderia glumae*, causes significant yield loss throughout the southern United States when it occurs during unusually hot weather conditions. No cultivars showing complete resistant have been identified for this disease worldwide. However, our research on this disease has confirmed the presence of significant partial resistance in the medium-grain rice variety Jupiter. The intent of this study was to identify the molecular mechanisms of partial resistance in Jupiter rice, in comparison with the susceptible rice cultivar Trenasse, using microarray analysis by Affymetrix GeneChip. Gene expression data indicated that 2294 genes were significantly (P = 0.05) up regulated 2 days after inoculation of Jupiter rice with *B. glumae*, compared to 533 genes in the susceptible variety. Fold differences ranged from 2.1 to 1490 in Jupiter and 2.1 to 109 in Trenasse. Important genes over-expressed in Jupiter were seed development protein genes (glutelin, prolamin and globulin), defense related genes (defensin) and genes involved in signal transduction, starch metabolism, transcription regulation and other cellular activities. Results were further validated using Real-time PCR analyses with selected genes. Knowledge of the molecular mechanisms of the partial resistance exhibited in Jupiter will assist in increasing the efficiency of developing varieties resistant to BPB in breeding programs.

Challenges in the management of plant diseases in the Northern Mariana Islands
D. NANDWANI (1), J. Tenorio (1)
(1) Northern Marianas College
Phytopathology 98:S112

Plant diseases are the main hindrance in the agriculture and causes significant losses to the food crops in the Northern Mariana Islands, though soil and other growing conditions are conducive for the farming. Planting traditionally from the old crop spreads the soil born diseases to the next generation and recent introduction of major diseases such as Panama wilt, Banana bunchy top, Taro leaf blight, Anthracnose, Phytophthora, Papaya ring spot virus have affected severely important vegetables, food and fruit crops. To meet the challenges and overcome constraints associated with diseases, the Northern Marianas College Cooperative Research, Extension and Education Service have significantly delivered several disease management programs in recent years to assist farming community in the Commonwealth. Modern scientific methods such as tissue culture techniques for the production of disease free and quality propagating materials and modern disease diagnostic tools like Enzyme Linked Immunosorbent Assays have been successfully implemented for the diseases diagnosis. Rouging or removal of infected plants, sanitation, and use of resistant varieties have been practiced to control and further spread of the diseases. Chemical control is used as last alternative and that is also very limited in use as not many fungicides or other chemicals available in the islands due to strict Government regulations. Shortage of quality planting material, high labor and management cost, dwindling economy and high cost of chemicals are the few constraints in the Commonwealth faced by the farming community. Several new varieties in food and fruit crops from certified institutions have been introduced to the Commonwealth successfully to produce quality and disease free planting material. The selected varieties of crops identified as the economically and culturally important with superior characteristics e.g. proven resistance to insect pest and devastating diseases, high yield and agronomic characters in the region.

Why do arbuscular mycorrhizal fungi form hyphal platoons?
G. NASIM (1), R. Bajwa (1)
(1) University of the Punjab, Lahore, Pakistan
Phytopathology 98:S112

Arbuscular Mycorrhizal (AM) fungi belong to the order Glomales (recently raised to the level of a division, Glomeromycota) is a small group of 6 genera ans 160 species. However, these fungi form mutualistic symbiotic relationship with over 95% of plant species. The literature is now ample for conforming the presence of AM structures in non-root portions also, like scale-like leaves, seed testa, organic matter, decaying leaves and peanut pegs etc. The hyphae are aseptate but occasionally form septa at maturity. These fungi may form platoons of mats of septate hyphae intra or extramatrically. When associated with non root portions like scale leaves, epidermis of rhizomes, corms or bulbs etc. or testa of seeds buried in soil, these hyphal mats are considered analogous to Arbuscules and act as the sites of bilateral exchange materials due largely to enormous surface area. Further more these are the sites from where the spores or the sporocarps develop as is being reported in the case of Sclerocystis pakistanica in the scale leaves of Chlorophytum commosum and Zephyranthus citrina, Glomus mosseae, G. fasciculatum and G. monosporum in the scales of and decaying sheathing leaves of wheat and Glomus intraradices and G. diaphanum in the mature roots of some of the Transgenic fluorescent tobacco lines. The present paper highlights the steps involved in the formation of hyphal congregations or platoons in association with these plant portions and their possible role in nutrient uptake and serving as propagules for vegetative reproduction of AM fungi.

A holistic approach to control potato late blight in organic production system in Parana, Brazil
N. NAZARENO (2), A. S. Pereira (1), C. B. Medeiros (1)
(1) Embrapa, Pelotas, RS, Brazil; (2) IAPAR, Curitiba, PR, Brazil
Phytopathology 98:S112

Organic potato production in the State of Parana, Southern Brazil is rising steadily. Late blight (LB) caused by *Phytophthora infestans* is the greatest concern among organic farmers as environmental conditions are highly favorable to the disease. Potato cultivars used by farmers are of European origin and highly susceptible to LB so that epidemics are common and devastating unless chemical control measures are taken. Although Bordeaux mixture is still commonly used, it will be soon banned. With regard to cultural controls, crop rotation is already practiced widely in Parana for control of other diseases. Regrettably, this is ineffective against LB since infected volunteer potato plants within rotational crops such as corn and field beans frequently serve as inoculum source. Roguing is recommended to limit volunteer plants. During the past decade, major emphasis has been on the development of LB resistant cultivars in a multi-institutional project involving IAPAR and EMBRAPA, State and National Research Institutions, respectively. A new potato clone resistant to LB has been tested in organic farms since 2003 and was recently released as Cristina by IAPAR. It is tolerant to common scab and adequate for the fresh market. Ana, another resistant cultivar lately released by EMBRAPA is high yielding and has good frying quality. Other resistant clones are being selected to fit the system. Overall LB control measures in organic system in Parana will be discussed.

Arabidopsis defense pathways activated by *Bacillus mojavensis* isolate 203-7 and *B. mycoides* isolate BmJ
O. T. NEHER (1), B. J. Jacobsen (1)
(1) Montana State University, Bozeman, MT, USA
Phytopathology 98:S112

An *Arabidopsis thaliana* – *Botrytis cinerea* pathosystem was used to investigate the plant defense pathways activated by *Bacillus mojavensis* isolate 203-7 and *B. mycoides* isolate BmJ. *A. thaliana* wild type and mutants jar1-1, npr1-5, ndr1-1/npr1-2, and NahG, were induced by application of washed cells (10^8 cfu/ml) of 203-7 or BmJ, sterile dH_2O, or chemical inducers, including Acibenzolar-S-methyl, methyl jasmonate, or probenazole. 6 days post induction 3 individual leaves per plant were challenge inoculated with 5 µl droplets of a *B. cinerea* spore solution (10^5 cfu/ml) and apoplastic proteins were harvested from separatly induced plants. 7 days post challenge inoculation disease severity was rated on a 0–5 scale. Both bacilli reduced disease severity on NahG but provide no disease reduction on jar1-1 ($P \leq$ 0.05) indicating that induction was salicylic acid independent but jasmonic acid dependent. NPR1 gene mutants (npr1-5, ndr1-1/npr1-2) showed enhanced disease symptoms for both bacilli treatments, but where significantly different ($P \leq$ 0.05) from jar1-1. Enzyme assays confirmed induction of chitinase, beta-1,3-glucanase, and superoxide dismutase (SOD) by 203-7 in npr1-5. 203-7 significantly increased ($P <$ 0.05) chitinase activity in NahG, but did not increase the levels of glucanase or SOD compared to the sterile dH_2O control. BmJ induction did not increase the activity of all tested enzymes in wild type Arabidopsis and npr1-5 mutant plants. Only NahG mutants sprayed with BmJ showed increased expression of chitinase, glucanase, and SOD but were not statistically ($P \leq$ 0.05) different from the sterile dH_2O control. Chemical inducers performed as expected and were significantly ($P \leq$ 0.05) different from the sterile dH_2O control.

Response of selected woody species to inoculation with *Phytophthora citricola* and *P. cactorum* from European beech using multiple inoculation methods
A. H. NELSON (1), J. E. Weiland (2), G. W. Hudler (1)
(1) Department of Plant Pathology and Plant Microbe Biology, Cornell University, Ithaca, NY, USA; (2) USDA ARS, Corvallis, OR, USA
Phytopathology 98:S112

Phytophthora citricola and *P. cactorum* are important cosmopolitan plant pathogens with wide host ranges. Both species have recently been identified as the cause of bleeding canker of European beech (*Fagus sylvatica*) in the northeastern United States, but whether isolates from European beech had the

ability to cause disease on other woody plant species in landscapes or forests was unclear. The objective of this research was to develop and apply methods to test pathogenicity of *Phytophthora* species from European beech on a variety of woody hosts. Using two isolates of *P. cactorum* and five isolates of *P. citricola*, greenhouse-grown seedlings were inoculated via one of three methods: colonized agar plugs were placed on the abaxial surface of detached leaves, zoospore suspensions were placed on wounded leaves still attached to the plant, and colonized agar plugs were inserted beneath the bark of the stem. European beech developed symptoms using all three methods. Expansion of these methods to nine additional plant species indicated varying susceptibility, with American elm, white ash, sweet birch, sugar maple and common lilac showing leaf susceptibility, while only birch and lilac showed stem susceptibility. The ability of the pathogens to progress from the leaf into the stem - found in European beech, lilac and birch - corresponded to general susceptibility of the stem.

Pre-emergence damping-off of soybean caused by *Fusarium solani*
B. D. NELSON (1)
(1) Dept. Plant Pathology, NDSU, Fargo, ND
Phytopathology 98:S113

Fusarium solani causes a root rot of soybean and is often associated with diseased soybean seedlings in the Red River Valley of Minnesota and North Dakota. To evaluate the role of *F. solani* as a seedling pathogen, the association between soil inoculum levels and seedling damping-off was investigated using two isolates, 115-3 and 31-2, from soybean. An Embden fine sandy loam was autoclaved and fresh conidia were mixed into the soil at 0, 25, 75, 100 and 500×10^3 and 1×10^6 conidia/g of air dried soil. Experiments with the same inoculum levels were also conducted in non-autoclaved soil with isolate 115-3. 'Sargent' soybean was planted in pots with four replications of 20 seeds each and the experiments were maintained in the greenhouse and repeated. Emergence and survival were determined at 14 and 21 days, respectively. In all experiments, *F. solani* significantly ($P = 0.05$) reduced emergence at all soil inoculum levels, but the amount of reduction varied between experiments and inoculum levels. At 25×10^3 conida/g soil the reduction in emergence averaged 46% and 62% for 115-3 and 31-2, respectively, in the autoclaved soil, and 66% in the non-autoclaved soil. There was no significant amount of post-emergence damping off within 21 days of planting. This data demonstrates that *F. solani* is one of the pathogens involved in the soybean seedling disease complex observed in the Red River Valley.

Seasonal fluctuations in *Cronartium ribicola* on *Ribes* related to rainfall
M. NEWCOMB (1), D. I. Rouse (1)
(1) Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI
Phytopathology 98:S113

For *Cronartium ribicola* and many heteroecious rusts the initial inoculum for disease on one host group is directly related to the pathogen's progression on the alternate host. The primary inoculum (basidiospores) for white pine blister rust results from disease on *Ribes* hosts. In North America the *Ribes* that are colonized by *C. ribicola* are generally wild plants. The objective of this study was to measure numbers of infectious rust lesions on a single *Ribes* species over the season in order to statistically summarize disease and associated environmental or forcing variables. Numbers of living (potentially infectious) lesions were counted 31 times from May through October on naturally-established *R. missouriense* shrubs in southern Wisconsin. Daily rainfall was recorded on-site and additional meteorological variables were recorded at a nearby station. Total numbers of rust lesions at each of two sites increased and decreased repeatedly during the season. A significant positive correlation was found between lagged rainfall and the change in numbers of living rust lesions between observations. The correlation with rainfall will be tested again in 2008. Final results will contribute empirical relationships between environmental factors and rust dynamics on *Ribes*. Knowledge of these relationships may advance our understanding of the pathosystem and in turn contribute to our understanding of inoculum for infection of white pines.

Explaining the association between apple tree stress and resistance to the fire blight bacterium *Erwinia amylovora*
H. K. NGUGI (2), D. K. Singh (1), B. L. Lehman (2), J. W. Travis (2), T. W. McNellis (1)
(1) The Department of Plant Pathology, 207 Buckhout Laboratory, Pennsylvania State University, University Park, PA 16802, USA; (2) The Department of Plant Pathology, 290 University Drive, Biglerville, PA 17307, USA
Phytopathology 98:S113

Fire blight caused by *Erwinia amylovora* remains an important disease limiting the productivity of apples in the U.S. In a survey of orchards during a fire blight outbreak in 2006, we documented a highly significant ($Chi^2 = 24.48$; $P < 0.0001$) association between tree vigor and disease severity. Trees with stress symptoms had little or no disease. The physiological and molecular mechanisms underlying this association were investigated using greenhouse-maintained, potted apple trees treated with either acibenzolar-S-methyl (ASM) or prohexadione-calcium (ProCa), or grown in a sandy potting mix with little water and no fertilizer (abiotic stress). All treatments resulted in a reduced average shoot length, which is an indicator of tree vigor, and a lower fire blight severity upon inoculation relative to untreated, unstressed control trees. Apical shoots of treated and stressed trees, excised at the time of *E. amylovora* inoculation, had significantly ($P < 0.05$) lower levels of total phenolic compounds and flavanols ($P < 0.001$) but not sorbitol ($P = 0.785$), compared to control, unstressed trees. All treatments increased the expression of pathogenesis-related genes *PR1a*, *PR2*, *PR5*, *PR8*, and *PR10*, relative to control, unstressed trees, suggesting that ASM, ProCa, and abiotic stress induce systemic acquired resistance in apple trees. Trees treated with ProCa exhibited the highest *PR* gene expression. We conclude that abiotic stress and prohexadion calcium protect apple trees from fire blight at least in part through induction of systemic acquired resistance.

Results of a survey of viruses found in peanuts in Georgia
C. NISCHWITZ (1), S. Mullis (1), R. Gitaitis (1)
(1) Department of Plant Pathology, Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31793-0748, USA
Phytopathology 98:S113

For the past several years the most common virus infecting peanuts in Georgia, the leading peanut producing state in the U.S., has been *Tomato spotted wilt virus* (TSWV). However, recently some peanut plant symptoms were suspected as having a different etiology. During the summer of 2007, leaflets from over 1,500 peanut plants with virus-like symptoms were collected in a survey from different peanut growing areas in Georgia. Each sample was tested with ELISA for the presence of TSWV, potyviruses and CMV. Veracity of selected samples testing positive with ELISA were verified with RT-PCR. Samples testing negative by ELISA, but having possible symptoms described for known peanut viruses not currently in Georgia, were tested with RT-PCR using various but known primers. Of the peanut samples testing positive with ELISA, 75% tested positive for TSWV, 52% tested positive for potyviruses and 1.2% tested positive for CMV, indicating mixed infections occurred in some samples. Identification of specific potyviruses with RT-PCR so far have confirmed the presence of *Zucchini yellow mosaic virus*, *Peanut mottle virus* and *Peanut stripe virus*.

Use of mechanistic simulation models to predict disease intensity of Fusarium head blight and deoxynivalenol concentration
M. NITA (1), E. De Wolf (1), L. Madden (5), P. Paul (5), G. Shaner (3), T. Adhikari (2), S. Ali (2), J. Stein (4), L. Osborn (4), S. Wegulo (6)
(1) Kansas State University, Manhattan, KS, USA; (2) North Dakota State University, Fargo, ND, USA; (3) Purdue University, West Lafayette, IN, USA; (4) South Dakota State University, Brookings, SD, USA; (5) The Ohio State University, Wooster, OH, USA; (6) University of Nebraska - Lincoln, Lincoln, NE, USA
Phytopathology 98:S113

Fusarium Head Blight (FHB) of wheat and Barley is an important plant disease that causes loss of millions of dollars in an epidemic year. A group of fungi can cause the disease, and in the North America, Fusarium graminearum (teleomorph: *Gibberella zeae*) is considered as a primary pathogen. Shrunken infected kernels and micotoxins (deoxynivanenol or DON) are primary causes of economic loss. In order to prevent the outbreak of the disease, a web-based FHB forecasting tool (http://www.wheatscab.psu.edu/) was developed. As a part of this forecasting effort, mechanistic modeling approach has been employed. An object-oriented language STELLA (isee systems) was utilized to create mechanistic simulation models for FHB and DON based on the results of past studies on disease development and pathogen biology. Major steps in the disease cycle, such as perithecia development and infection events, were expressed as differential equations that use environmental conditions as input variables. Several candidate models have been developed with different scope of interest, and results from one of candidate models will be discussed. This model estimates a distribution of Fusarium damaged kernels and DON accumulation among heads of wheat, and incorporates theoretical functions of Type I and Type II resistant genes. Model development process and results from model calibration using historical weather and disease data will be presented.

Decline in viability of *Gibberella zeae* ascospores after exposure to the solar radiation
M. NITA (1), E. De Wolf (1), S. Isard (2)
(1) Kansas State University, Manhattan, KS, USA; (2) Pennsylvania State University, University Park, PA
Phytopathology 98:S113

Ascospores of *Gibberella zeae* are considered to be an epidemiologically important type of inoculum for Fusarium head blight of wheat and barley. The

objectives of this study were to evaluate the effects of solar radiation, temperature, and relative humidity on the survival of ascospores in the environment. Experiments evaluating the effects of environment on spore survival were conducted at Rock Springs, PA and Manhattan, KS. In each experiment, ascospores were placed on glass Petri dishes and exposed to solar radiation or shaded conditions for predetermined lengths of time. The temperature of the exposed and shaded spores was kept constant by allowing the dishes to contact a circulating source of water. Following exposure, the spores were placed on water agar and incubated for 8-10 h. The percentage of germinating spores was expressed as a ratio of the initial germination of spores for that experimental run. Regression analysis suggests that total solar radiation and the dose of UV radiation significantly impacted spore survival, but that temperature and relative humidity may also be important variables. The dose of radiation resulting in 100% ascospore mortality was 20 and 60MJ/m^2 at the KS and PA location, respectively. Differences between the locations may be explained in part by differences in the isolate considered and range of temperatures experienced during the exposure periods.

Application of mycelial compatibility grouping in studying intra-field spread of *Sclerotinia trifoliorum* in a chickpea field

E. N. NJAMBERE (3), W. Chen (1), C. Frate (2), S. Temple (2)
(1) USDA ARS, Pullman, WA, USA; (2) University of California, Davis, CA, USA; (3) Washington State University, Pullman, WA, USA
Phytopathology 98:S114

Sclerotinia trifoliorum causes stem and crown rot of chickpea and other leguminous forage crops. In order to study population structure and patterns of in-field spread, isolates of *S. trifoliorum* were intensively sampled from a chickpea field near Five Point, California, in 2006. All diseased plants were result from crown infection. A total of 55 sclerotial isolates were obtained from 36 disease foci, and each isolate was obtained from a different diseased plant. The isolates were tested for mycelial compatibility groups (MCGs) and polymorphisms within the nuclear small subunit rDNA. Twenty-seven MCGs were found among the 55 isolates. The largest MCG contained 22 isolates, another MCG contained five isolates and three other MCGs each contained two isolates. The remaining 22 isolates each belonged to a different MCG. Three rDNA haplotypes were detected by analysing four polymorphic sites in the rDNA. Isolates of the same MCG belonged to the same rDNA haplotype except one isolate. Although more than one MCG were found within disease foci, many of the isolates collected from adjacent plants belonged to the same MCG, suggesting that spreading by mycelial growth from adjacent plants is common. Also a single MCG was found dominating the population, suggesting that this MCG may be an early introduction or have a selective advantage in infecting chickpea plants.

Comparative analysis of infection of broccoli and cauliflower by a GFP-tagged *Verticillium dahliae* isolate

S. NJOROGE (2), S. Park (1), S. Kang (1), K. Subbarao (2)
(1) Department of Plant Pathology, Pennsylvania State University; (2) Department of Plant Pathology, University of California, Davis
Phytopathology 98:S114

The response of broccoli cv. Patriot and cauliflower cv. White Rock to infection by a gfp-tagged *Verticillium dahliae* isolate was compared. Seedlings were inoculated 1, 2, 3, 4, 5, and 6 wk after emergence by drenching soil surrounding seedlings with 5-ml aliquots of 10^6 conidia per ml suspension. Control plants were treated with sterile distilled water. Infection was followed using epifluorescence and confocal laser scanning microscopy. Control plants did not reveal the presence of *V. dahliae* at any time. Two to 3 wk after inoculation, differences in colonization intensity were apparent in broccoli and cauliflower plants inoculated at similar times as well as at different seedling ages. Cauliflower plants inoculated one week after emergence wilted and exhibited extensive vascular colonization and collapsed lateral roots. In contrast, no broccoli plants inoculated at any time exhibited wilting. *V. dahliae* was observed in the stem vascular tissue of broccoli plants inoculated at 1, 2, and 3 wk only. No colonies were observed in the above ground vascular tissues of broccoli seedlings inoculated at 4, 5, and 6 wk. *V. dahliae* was observed consistently in both hypocotyl and epicotyl vascular tissues of mature cauliflower plants. These observations provide additional evidence to the resistance to *V. dahliae* infection exhibited by broccoli. Other age-related factors that may contribute to the resistance in broccoli will be discussed.

Identification of gene-specific markers for resistance to *Erwinia amylovora* (fire blight) in *Malus* (apple) by a functional genomics approach

J. L. NORELLI (6), S. E. Gardiner (3), M. Malnoy (1), H. S. Aldwinckle (1), R. E. Farrell (4), M. B. Horner (2), J. Celton (3), A. M. Baldo (5), D. R. Bowatta (3), C. M. Carlisle (3), D. A. Lalli (6), C. L. Bassett (6), M. E. Wisniewski (6)
(1) Cornell University, Geneva, NY, USA; (2) HortResearch, Havelock North, New Zealand; (3) HortResearch, Palmerston North, New Zealand; (4) Pennsylvania State University, York, PA, USA; (5) USDA-ARS, Geneva, NY, USA; (6) USDA-ARS, Kearneysville, WV, USA
Phytopathology 98:S114

Fire blight, caused by *Erwinia amylovora* (*Ea*), is a destructive disease of apple (*Malus*), pear (*Pyrus*) and other plants in the rose family (Rosaceae). 650 expressed sequence tags (ESTs) associated with fire blight were identified from *Ea*-challenged apple leaf tissue by suppression subtractive hybridization (SSH) and cDNA-AFLP analysis. ESTs were ranked for their potential impact on resistance based on bioinformatics and inferences drawn from model systems. Simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers derived from highly ranked fire blight-associated ESTs were mapped in a 'M.9' × 'Robusta 5' population in which a major QTL for fire blight resistance has been located on Linkage Group 03. Markers for heat shock protein 90 (Hsp81-2), a secretory class III peroxidase and a serine/threonine-protein kinase mapped to the LG03 fire blight resistance QTL and reduced its size from 12cM to 4cM. Markers for a "putative disease resistance protein" (NCBI AY347778) and Skp1 (SCF-type E3 ubiquitin ligase) mapped to positions corresponding to the location of two QTLs reported in other populations (Calenge et al. 2005, Khan et al. 2006). To date, of 28 candidate fire blight resistance gene markers that have been mapped, 6 have co-located to or near known fire blight resistance QTLs. This research will facilitate new methods of marker-assisted selection to efficiently breed superior apple cultivars with fire blight resistance.

Comparing the genetic diversity of *cucumber mosaic virus* (CMV) in snap bean and pepper: Implications for locally specific inoculums

S. NOURI (1), T. German (1), R. Groves (1)
(1) University of Wisconsin-Madison, Madison, WI 53706, USA
Phytopathology 98:S114

Cucumber mosaic virus (CMV) is a tripartite, positive-sense RNA plant virus in the genus Cucumovirus, family *Bromoviridae*. This virus is one of the most economically important viruses affecting several crop plant species and it has one the widest host ranges among the characterized viruses. Recently, snap bean (*Phaseolus vulgaris* L.) and pepper (*Capsicum annuum* L.) crops in Wisconsin have experienced significant increases in incidence and crop losses associated with infection of this virus. This increase has anecdotally been linked to the recent introduction and establishment of the soybean aphid (*Aphis glycines* Matsumura) in the upper Midwest region. Presumably, the unique population biology and dispersal of this species has changed both the spatial arrangement and temporal movement patterns previously observed with respect to CMV in the region. We propose to compare the genetic diversity of virus populations present in affected crops over years, within and among locations, between affected crops, and finally within dispersing aphid vectors. CMV has three RNA and five open reading frames (ORF's). Reassortment between RNAs and recombination in the 3′ and 5′ nontranslated regions and between ORFs 3a and 3b have been demonstrated to contribute to CMV evolution. To complete these diversity studies, we will examine sequence heterogeneity in all six regions of the genome. To date, a subset of plant samples representing CMV-affected snap bean and pepper have been sequenced. RNA extraction, RT-PCR with specific primer 2b, PCR product purification, cloning of PCR product with pGEM-T Easy Vector System, plasmid purification and finally sequencing was done according to manufacturer's instruction. Sequence alignments were performed using CLUSTAL X and phylogenetic trees were generated using PAUP. Preliminary results illustrate close homology among selected sequences within locations and also affected crops.

Massively parallel sequencing of small RNAs from the rice blast fungus, *Magnaporthe grisea*

C. C. NUNES (2), M. Gowda (2), F. Chen (1), S. Meng (2), D. Brown (2), T. K. Mitchell (3), R. A. Dean (2)
(1) US DOE Joint Genome Institute, Walnut Creek, CA, USA; (2) North Carolina State University, Raleigh, NC, USA; (3) The Ohio State University, Columbus, OH, USA
Phytopathology 98:S114

High-throughput sequencing has been used to reveal novel functional regulatory RNAs in eukaryotes. MicroRNAs (miRNAs) and small interfering RNAs (siRNA) are the most common classes of non coding small RNA species. In fungi, a detailed investigation of small RNAs has not been undertaking to-date. Here, we used *Magnaporthe grisea*, the causal agent of rice blast, to clone and sequence small RNAs using RNA-ligation mediated protocol followed by 454 sequencing. We generated small RNA libraries with 318,454 and 343,304 reads from mycelia and appressoria, respectively. Preliminary analysis shows that small RNAs are highly diverse in sequence particularly at the 5′ and 3′ regions. Currently, we are characterizing miRNA/siRNAs which are differentially expressed in mycelia and

appressoria. Our main goal is to elucidate the role of miRNA/siRNAs during *M. grisea* appressorium development and pathogenesis.

Rapid assay for the on-site detection of potato pathogens
T. Oberhänsli (1), D. Altenbach (1), P. Munch (1), K. Maier (1), W. BITTERLIN (1)
(1) BIOREBA AG, Chr. Merian-Ring 7, CH-4153 Reinach BL1, Switzerland
Phytopathology 98:S115

Sanitary selection is an important factor in the (preventive) control of viral and other plant diseases. To maintain high crop quality, growers and inspectors need reliable diagnostic tools for a fast on-site detection of plant pathogens. Specific monoclonal antibodies, previously validated in double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA), were selected for the development of AgriStrip rapid assays (lateral flow devices, based on immunochromatography) for the detection of *Spongospora subterranea* f. sp. *subterranea* (Sss, powdery scab) and Potato virus Y (PVY), two important potato pathogens. Sensitivity and speed of detection of the AgriStrip assays for Sss and PVY were determined using serial dilutions of extracts from infected and healthy plant tissues, and compared to the traditional laboratory method DAS-ELISA. The sensitivity of both methods was essentially in the same range (detection until tissue dilutions of 1:320 w/v). The results of the AgriStrip lateral flow assay was obtained within minutes, whereas two days were needed for the laboratory assay DAS-ELISA. The rapid assay was more prone to influences of the matrix of plant extracts, which led to some green background reaction at high tissue concentration. This effect could be reduced with appropriate sample dilution of 1:80 w/v. Due to its performance, speed and ease of use, the AgriStrip is a valuable and useful diagnostic tool for the on-site detection of pathogens without any need of laboratory infrastructure, thus suitable for field application.

A rapid diagnostic tool for detecting benzimidazole resistance in *Cercospora beticola*, the causal agent of Cercospora leaf spot in sugarbeet
J. O. OBUYA (2), L. E. Hanson (1), W. L. Stump (2), G. D. Franc (2)
(1) USDA-ARS SBRU, 494 PSSB, Michigan State University, East Lansing, MI 48824-1325, USA; (2) University of Wyoming, Laramie, WY, USA
Phytopathology 98:S115

Resistance in *Cercospora beticola* to benzimidazole fungicides was selected in many sugar beet regions of the world due to extensive use of this fungicide class. Practical resistance to benzimidazole in populations of *C. beticola* was first reported in Greece in 1973. Resistance has subsequently been found in the majority of the growing areas worldwide, including *C. beticola* populations resistant to benzimidazole in the upper Midwest and in the High Plains region of the United States. Although reliance on benzimidazole use is reduced, it sometimes is still used in combination with other fungicide classes for *C. beticola* suppression. Rapid diagnostic methods are needed for detecting benzimidazole resistance in field isolates, with the goal of quickly providing information to producers pertaining to fungicide selection and disease management. Current methods require several weeks, which is too long to be used in making management decisions. Resistance to benzimidazoles can be due to several potential single nucleotide polymorphisms in the beta-tubulin gene. Based on characteristics of the predominant mutation detected in the High Plains pathogen populations, a diagnostic tool was developed that utilizes a combination of polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The same mutation has been detected in isolates from other U.S. growing regions. Thus, this method may be useful in many production areas. This diagnostic tool enables rapid discrimination between benzimidazole resistant and benzimidazole sensitive isolates of C. beticola.

Survey, evaluation and molecular characterization of Nigerian native fungus for potential biocontrol of water hyacinth
W. O. OKUNOWO (1), G. O. Gbenle (1), A. A. Osuntoki (1), A. A. Adekunle (2)
(1) Dept. of Biochemistry, College of Medicine, University of Lagos, Lagos State, Nigeria; (2) Dept. of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos State, Nigeria
Phytopathology 98:S115

The use of chemical herbicide in the control of aquatic weeds constitutes not only environmental problem but the cost and the non-specific nature calls for the use of bio-agents. Some water bodies in Lagos State Nigeria were surveyed to collect diseased water hyacinth (*E. crassipes*). The fungi present in the diseased tissue were isolated and identified as: *A. niger*, *A. flavus*, *Penicillium sp.*, *C. pallescens*, *F. solani* and *Myrothecium* sp. The phytopathogenicity on fresh non-diseased water hyacinth and some crops were examined. All the isolates except a strain of *Myrothecium* sp. were unable to induce disease symptoms on healthy plants. Necrosis was observed on the healthy plant 3 days post inoculation with 1 × 106 spores/ml *Myrothecium* sp. The leaves and the petioles were observed withered at the end of the third week. The Disease Incidence (DI) and the Mean Disease Severity (MDS) was 100% and 8.67 respectively, on day 24. None of these isolates inflicted a disease symptom on the 28 crops tested. Molecular analysis of the ITS rDNA of the water hyacinth pathogenic isolate showed >98% homology to authenticated sequences of *M. roridum*, and 99% homology to a strain identified as the closely related species *M. carmichaelii*. The isolate; *Myrothecium roridum* Tode (IMI 394934) possesses the level of virulence needed to be a potential mycoherbicide for use in the management of water hyacinth.

Investigative study of *M. roridum* toxin on water hyacinth
W. O. OKUNOWO (1), G. O. Gbenle (1), A. A. Osuntoki (1), A. A. Adekunle (2)
(1) Dept. of Biochemistry, College of Medicine, University of Lagos, Lagos State, Nigeria; (2) Dept. of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos State, Nigeria
Phytopathology 98:S115

The use of phytotoxin in the management of weeds is most promising as against the use of synthetic chemical herbicides with their health implications and the use of microbial agents which are sensitive to environmental conditions. An investigation was conducted on the production of phytotoxin using a fungus; *Myrothecium roridum* Tode (IMI 394934) isolated from diseased water hyacinth plants collected from Badagry Creeks and Ogun river in Lagos State Nigeria. The effects of various parameters were also investigated on both the phytotoxin production and the biological activity. The phytotoxin produced by the fungus caused necrosis and cell death on water hyacinth leaf three days post toxin infiltration. The phytotoxin production by this isolate was dependent on light. The amount of toxin produced was highest at pH 4.5, potato carrot broth and a formulated water hyacinth leaf broth enhanced the production than other media types. The best carbon source and nitrogen source were xylose and glutamine respectively. The phytotoxin was thermostable, and the biological activity was pH dependent and independent of photoperiod. The metabolite possesses the level of potency needed to be effective herbicidal agent hence; we consider it to be suitable herbicide for use in the management of water hyacinth in Nigeria.

Enhancing potato system sustainability: Microclimate, early blight and late blight potential
M. OLANYA (1), C. W. Honeycutt (1), R. P. Larkin (1), T. S. Griffin (1), Z. He (1), J. Halloran (1)
(1) USDA-ARS, NEPSWL, Orono, ME, USA
Phytopathology 98:S115

Crop and soil management may modify canopy and below ground microclimate; however, their effects on potential development and control of early and late blight are not well documented. Crop management systems (Status Quo (SQ), Soil Conserving (SC), Soil Improving (SI), Disease Suppressive (DS), and continuous potato (PP)) were evaluated for their effects on microclimate, early blight potential and late blight potential under irrigated and rainfed conditions. In 2006 and 2007, microclimatic data were continuously recorded with a data logger deployed at the canopy level. Early blight was determined by visual assessment of symptoms and late blight potential was evaluated based on simulation analysis. In both years, very little variation in mean relative humidity, ambient temperature or soil temperature was detected among treatments. In 2006, soil water content was significantly higher in irrigated than in rainfed treatments in August, and ranged from –53 to –58 kPa (irrigated) compared to –90 to –130 kPa (rainfed) in DS, SQ, SC, and SI treatments. Early blight incidence and lesion numbers varied among cropping systems and between years. Disease incidence ranged from 31–64% (2006) and 12–43% (2007), and was significantly higher in PP compared to DS, SQ, SC, and SI systems. Simulated late blight development differed between years but not among cropping systems. Cropping system impacted early blight incidence, as demonstrated by more early blight detected in the PP system, compared to other systems. However, small differences in microclimate and late blight indices suggest that the cropping systems evaluated had relatively little impact on late blight potential.

Influence of El Niño Southern Oscillation (ENSO) on tomato spotted wilt incidence and peanut yield
R. O. OLATINWO (1), J. O. Paz (1), A. K. Culbreath (2), R. C. Kemerait (2), G. Hoogenboom (1)
(1) Department of Biological and Agricultural Engineering, University of Georgia, Griffin, GA 30223, USA; (2) Department of Plant Pathology, University of Georgia, Tifton, GA 31793, USA
Phytopathology 98:S115

Spotted wilt caused by Tomato spotted wilt virus (TSWV) has resulted in significant economic losses to peanut growers in the southeastern United States since it was first observed in 1986. The virus is transmitted by western flower thrips Frankliniella occidentalis and tobacco thrips, Frankliniella fusca. Spotted wilt incidence and vector populations may vary greatly from one year to the next. The goal of this study was to improve peanut yield by

understanding the impact of climate variability on spotted wilt incidence. The specific objective was to examine the influence of El Niño Southern Oscillation (ENSO) on spotted wilt incidence and peanut yield. Our analysis was based on field experiments conducted between 1998 and 2005 at two locations (Tifton, Georgia and Marianna, Florida). The incidence of spotted wilt (%) in 'Georgia Green' variety and the corresponding yield were recorded. Each season was categorized by ENSO (El Niño, Neutral or La Niña) based on NOAA Climate Prediction Center ENSO classification scheme. The results showed a significant interaction between the ENSO, field location and spotted wilt incidence. The incidence of spotted wilt was significantly lower during La Niña compared with El Niño or Neutral. The highest yield was observed in Marianna during a La Niña and lowest yield in Tifton during a Neutral. Incidence of spotted wilt was lowest in Marianna during La Niña and highest in Tifton during Neutral. The results also showed a significant correlation between spotted wilt incidence and peanut yield. Incorporating findings from this study into the existing TWSV risk index could aid in making management decisions for reducing spotted wilt.

Sensitivity of *Phytophthora capsici* isolates to the carboxylic acid amides fungicides mandipropamid and dimethomorph

G. OLAYA (3), A. P. Keinath (1), P. D. Roberts (4), A. Tally (2)
(1) Clemson University, CREC, Charleston, SC; (2) Syngenta Crop Protection, Greensboro, NC; (3) Syngenta Crop Protection, Vero Beach, FL; (4) University of Florida, SWFREC, Immokalee, FL
Phytopathology 98:S116

Mandipropamid is a new fungicide effective against Phytophthora and downy mildew diseases. Mandipropamid is the active ingredient of Syngenta fungicide Revus® and belongs to a new chemical class of fungicides known as the carboxylic acid amides (CAA). The sensitivity to mandipropamid of *Phytophthora capsici* isolates was determined under *in vitro* conditions using a mycelial growth assay. Seventy-five *P. capsici* isolates were obtained from pepper (31), zucchini (16), squash (18), tomato (3), watermelon (4) pumpkin (2) and an unknown host (1) and from four geographical areas (FL, SC, IL, CA). The mandipropamid sensitivity distribution (ED_{50} values) ranged from 0.0056 to 0.0265 mg/L (range: 4.3X), with a geometric mean of 0.014 mg/L. The sensitivity of 40 isolates was also determined for dimethomorph, a fungicide also belonging to the CAA class. The ED_{50} values for dimethomorph ranged from 0.068 to 0.333 mg/L (range: 4.9X), with a geometric mean of 0.226 mg/L. The sensitivity data demonstrates that mandipropamid has a higher intrinsic activity against *P. capsici* compared to dimethomorph. The mandipropamid sensitivity data will provide a baseline for monitoring changes in sensitivity in populations of *P. capsici* after the 2008 commercial introduction of mandipropamid (Revus®) in the United States.

Sensitivity to azoxystrobin, difenoconazole and cyprodinil of *Alternaria* spp. isolates causing Alternaria leaf spot on almonds

G. OLAYA (2), R. Bounds (1), A. Tally (1)
(1) Syngenta Crop Protection, Greensboro, NC; (2) Syngenta Crop Protection, Vero Beach, FL
Phytopathology 98:S116

Fifty four isolates of *Alternaria* spp. were evaluated *in vitro* to determine their sensitivity to azoxystrobin, difenoconazole and cyprodinil. The isolates were collected from infected leaves obtained from an almond field in Kern County, California. A single azoxystrobin discriminatory dose of 10 mg/L was used to differentiate sensitive and resistant isolates. The frequency of azoxystrobin resistant isolates was high (49%). The sensitivity to difenoconazole and cyprodinil of 8 azoxystrobin sensitive and 27 resistant isolates was also determined *in vitro*. All the 35 isolates were sensitive to difenoconazole and cyprodinil. Sensitivities of *Alternaria* isolates to difenoconazole (ED_{50} values) ranged from 0.010 to 0.035 with a mean sensitivity of 0.022 mg/L. The cyproconazole sensitivity of the *Alternaria* isolates ranged from 0.001 to 0.011 with a mean sensitivity of 0.005 mg/L. It is clear that the control of Alternaria leaf spot on almonds could be reduced due to the high frequency of azoxystrobin resistant isolates. Azoxystrobin resistance in *Alternaria* also indicates that there is resistance to any other QoI fungicide use to the control this almond disease such as pyraclostrobin. *Alternaria* isolates sensitive and resistant to azoxystrobin were sensitive difenoconazole and cyprodinil. The introduction of these 2 fungicides to control *Alternaria* spp. of almonds and pistachios could improve disease control and diversify chemical control programs for Alternaria diseases with good resistance management practices.

Genetic variability within *Grapevine fanleaf virus* isolates in a naturally infected California vineyard

J. E. OLIVER (1), M. Fuchs (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, NYSAES, Geneva, NY, USA
Phytopathology 98:S116

Grapevine fanleaf virus (GFLV) causes fanleaf degeneration, the most important disease of grapevines worldwide. GFLV belongs to the genus *Nepovirus* in the family *Comoviridae*. It is specifically transmitted by the ectoparasitic nematode *Xiphinema index*. GFLV possesses a bipartite (+)ssRNA genome, which is expressed into two polyproteins that are cleaved into at least eight individual proteins. Due to its error-prone replication, GFLV possesses great potential for genetic variation. Information on the genetic variability of GFLV is available, but consists largely of data from isolates of diverse geographic origin and from two individual vineyards in Europe. Our objective was to examine the genetic variability between GFLV isolates in a naturally infected vineyard in California. GFLV-infected grapevines were identified by ELISA. Immunocapture-reverse transcription-PCR, cloning, and sequencing were used to determine nucleotide variability among GFLV isolates. Results will be presented and discussed with regard to improved construct designs for GFLV control through genetic engineering.

Characterization of *Phytophthora* in North Carolina greenhouse ornamentals

H. A. OLSON (1), M. Benson (1)
(1) North Carolina State University, Raleigh, NC, USA
Phytopathology 98:S116

Root rot, crown rot, and foliar blight, caused by species of *Phytophthora*, are common diseases on ornamental crops and are ongoing problems in greenhouse production. Greenhouse facilities in North Carolina were surveyed to expand the data from a 2001–2002 survey. Symptomatic plants were collected, and direct isolation from plant material was conducted on a selective medium. To date, *Phytophthora* isolates have been identified as *P. cryptogea*, *P. drechsleri*, *P. nicotianae*, and *P. tropicalis* using morphology and sequences of the ITS region. Incubation at 35°C has been used to distinguish *P. cryptogea* (no growth) and *P. drechsleri* (growth). Utility of this assay was evaluated with collected isolates. Results were inconclusive. Isolates of *P. cryptogea* did not grow; however, not all *P. drechsleri* isolates grew at 35°C. Mefenoxam sensitivity was tested using growth on mefenoxam-amended medium. Isolates of *P. cryptogea* and *P. tropicalis* were sensitive; whereas, *P. drechsleri* isolates were insensitive. Mefenoxam sensitivity of *P. nicotianae* ranged from sensitive to insensitive and depended on the geographic origin of the isolate. The *P. cryptogea* isolates from the 2001–2002 survey were characterized further by ITS and *cox* sequencing. Inconsistencies between the ITS RFLP, ITS sequence, and *cox* sequence identifications are being explored. In-depth characterization of the species present will further our understanding of the impact of *Phytophthora* on the floriculture industry in North Carolina.

The influence of phosphorus concentration on the development of Pythium root rot disease of seedling geranium

M. OMER (1), J. Locke (1), J. Frantz (1), C. Krause (1)
(1) USDA-ARS
Phytopathology 98:S116

In greenhouse production systems, growers may increase nutrient supply to meet production demands or decrease nutrient supply due to cost or environmental concerns. Only a few floriculture crops' response in different nutrient environments to a handful of diseases are well known. Seeding geraniums were planted in soilless media and fertilized with Hoagland's solution that contained a range of phosphorus concentrations, from 0.05 mM to 15 mM, and challenged with *Pythium ultimum* in an attempt to study the role of P in mediating disease stress. Disease severity was lowest in a P supply of 0.2 to 2.0 mM. An increase in P concentration from the concentration of 0.5 mM to 7.5 and 15 mM doubled disease severity. Lowering the P concentration to 0.05 mM resulted in a similar disease response as the highest P supply (P = 0.05). Plant biomass showed a similar trend with the highest and lowest P concentrations having similar shoot weight (P = 0.05). Data presented here showed that the use of Hoagland's solution containing 0.2 or 0.5 mM P would help in lessening the effect of *P. ultimum* on seedling geranium and in increasing plant vigor. This represents a decrease of P of nearly 60% from typical production fertility programs.

Effects of glucosinolates from brassicaceous plants on nematode populations

K. ONG (2), K. Steddom (3), J. L. Starr (1)
(1) Texas A&M University, College Station, TX; (2) Texas AgriLife Extension Service, Dallas, TX; (3) Texas AgriLife Extension Service, Overton, TX
Phytopathology 98:S116

Plants from the brassica family have been used as biofumigant agents in managing soilborne pathogens. We utilized several varieties of these plants in a sweet potato production system to evaluate their ability to suppress nematode population levels. Brassicas, used as winter cover crops, were destroyed and tilled under as green manures. Samples of the plants were frozen and later analyzed for concentration of two glucosinolates,

glucotropaeolin and sinigrin. Nematode populations were sampled prior to brassica incorporation, 4-weeks post incorporation and at sweet potato harvest. There were no significant differences in nematode population between treatments at 4-week post incorporation of the winter cover crop. However, regression analysis of the data suggested that the amount of sinigrin and glucotropaeolin in the cover crops could predict final nematode population at sweet potato harvest. A 0.22% decrease of ring nematode at the end of the growing season is predicted with 1 mmol sinigrin/m^2 increase. On the other hand, there is a 1.47% increase in stunt nematode population at the end of the growing season for every 1 mmol/m^2 increase of glucotropaeolin. Although glucosinolates are not expected to be in the soil for long periods of time, it may be one of several indicators to predict the trend of nematode population during the growing season.

Comparative effect of aqueous plant extracts in the control of storage fungi

C. ONYEANI (1), S. Osunlaja (1), O. Oworu (1), A. Joda (1)
(1) Dept. of Crop Production & Protection, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria
Phytopathology 98:S117

A study of the control of fungal pathogens on stored products using aqueous plant extracts was carried out in – vitro in an attempt to find suitable natural plant extracts for fungal control of storage fungi. Extracts of bark and leaves of Acalypha ciliata, Annona squamosa, Aloe vera, Azadirachta indica, Persea americana, Melia azedarach, Newbouldia laevis and Vernonia amygdalina were incorporated separately into agar medium in petri dishes and pure cultures of each fungal pathogens (Aspergillus Floavus, Aspergillus niger, Penicillium expansum and Rhizopus stolonifer) was placed at the center of separate Petri-dishes and incubated for 4 days at 25°C using complete Randomised design experimental layout. Analysis of variance and means separation for mycelial growth and sporulation inhibition at 5% level of indica and Vernonia amygdalina were as effective as benomyl in probability showed that Acalypha ciliata, Aloe vera, Azadirachta the control of Aspergillus flavus and penicillium expansum. Annona squamosa was as effective as benomyl in inhibiting Rhizopus stolonifer. Also, Annona squamosa and Aloe vera were singnificant in the inhibition of Aspergillus niger. Leaf extracts showed better inhibition in the overall result than bark extracts and these need to be evaluated in-vivo to further substantiate their effectiveness or otherwise.

Lack of genetic differentiation between *Puccinia triticina* collections from North and South America

M. E. ORDONEZ (1), J. A. Kolmer (1)
(1) ARS-Cereal Disease Laboratory
Phytopathology 98:S117

Isolates (265) of *Puccinia triticina* from Argentina, Brazil, Chile, Peru, Uruguay, Canada and the U.S. were tested for polymorphism at 23 simple sequence repeat (SSR) loci, and tested for virulence on 20 Thatcher near-isogenic lines of wheat. Grouping of the isolates through a Bayesian model-based clustering method with SSR allele frequencies separated the populations of North and South America into 10 groups. However, major groups of isolates with similar virulence phenotypes from both continents were not significantly differentiated based on SSR genotypes. Introduction of similar genotypes into both North and South America and/or migration of *P. triticina* between the two continents has likely occurred. Isolates between the two continents differ for virulence since the populations undergo mutation and are selected for virulence to the *Lr* resistance genes in the different wheat cultivars of each region. *P. triticina* populations in both continents had high levels of SSR heterozygosity and disequilibria typical of asexual populations.

Development of molecular markers for fine mapping of the *Rps*8 gene locus in soybean

M. A. ORTEGA (3), D. M. Tucker (1), G. Pfeifer (6), W. Pipatpongpinyo (3), S. Berry (3), D. Hyten (4), P. Cregan (4), R. Shoemaker (5), S. St. Martin (2), M. Maroof (1), A. E. Dorrance (3)
(1) Crop and Soil Environmental Science Dept., Virginia Tech, Blacksburg, VA; (2) The Ohio State University, Columbus, OH; (3) The Ohio State University, OARDC, Wooster, OH 44691; (4) USDA-ARS, Beltsville, MD; (5) USDA-ARS, Corn Insects and Crop Genetics Research, Iowa State University, Ames, IA; (6) USDA-ARS, Iowa State University, Ames, IA
Phytopathology 98:S117

Phytophthora sojae resistance gene *Rps*8 maps to Molecular Linkage Group (MLG) F (Chromosome 13). Our first objective was to identify SSR and SNP markers in this region. Additional markers would permit fine mapping of *Rps*8 and screening a PI399073 BAC library for the eventual cloning of the resistance gene. Sequences derived from Williams82 BAC clones, which were previously identified with markers from the composite map, and the clone gmw2-210K02 were used to identify 200 SSRs. In addition, four SNPs were developed from A186_1, Satt595, genes *Rpg*1b and *Hsp*176 as were 12 genic SNPs from the database. SSR and SNP markers which mapped close to *Rps*8 were aligned with the 7X whole genome sequence of soybean using BLAST. This process identified an additional 173 SSRs in 7 supercontigs. Three BC_4 Williams × PI399073 populations and a $F_{2:3}$ Hutcheson x PI399073 population were assayed with the new polymorphic markers. The majority of the markers in the region with *Rps*8 were distributed in 2 scaffolds of the whole genome sequence, spanning between 100 and 400kb. Four populations harvested autumn 2007, including $F_{3:4}$, $BC_2F_{2:3}$, $BC_3F_{2:3}$ Williams × PI399073, and a $F_{3:4}$ PI408211B × PI399073 were screened with the new set of markers. We have developed more than 30 markers in this region on MLG F identifying polymorphisms between the different parental genotypes.

Resistance of maize land races from Mexico to aflatoxin contamination

A. ORTEGA-BELTRAN (1), A. Ortega-Corona (2), M. Guerrero-Herrera (2), V. A. Vidal-Martinez (3), P. J. Cotty (4)
(1) Dept. of Plant Sciences, University of Arizona, Tucson, AZ; (2) SAGARPA-INIFAP-CIANO, Cd. Obregon, Sonora, Mexico; (3) SAGARPA-INIFAP-CIANO, Santian Ixcuintla, Nayarit, Mexico; (4) USDA-ARS, Dept. of Plant Sciences, University of Arizona, Tucson, AZ
Phytopathology 98:S117

Maize, the critical staple of billions, was domesticated in Mexico about 7000 years ago. Today, across rural Mexico, a great array of maize land races is harbored in a living library that has been passed between generations since before establishment of the modern state. These land races have been selected by ethnic groups over hundreds of generations for adaptation to diverse local climates and soil types. The great natural genetic diversity of maize land races in Mexico is an outstanding resource for development of maize cultivars with traits beneficial to human populations across the world. Maize is frequently contaminated with the highly carcinogenic aflatoxins by members of *Aspergillus* section Flavi. Variation among 34 maize land races in susceptibility to aflatoxin contamination was evaluated in laboratory tests. The land races were collected in 2006 and 2007 from two regions of Mexico. Tests were performed on surface disinfected living maize kernels at 31°C with kernel moisture adjusted to 25%. Both highly resistant and highly susceptible races exist in both regions. The four most resistant accumulated 88–93% less aflatoxin than the commercial hybrid control. These studies indicate that maize land races from Mexico are potential sources of resistance to both kernel rot and aflatoxin contamination caused by *A. flavus* and may contribute to the breeding of healthier food for humans and domesticated animals.

Resistance and tolerance to *Meloidogyne javanica* in *Colocasia esculenta* from Thailand, Vietnam, and Nepal

A. Ortiz (2), S. Miyasaka (1), J. Cho (3), B. SIPES (2)
(1) University of Hawaii, Hilo, HI, USA; (2) University of Hawaii, Honolulu, HI, USA; (3) University of Hawaii, Kula, HI, USA
Phytopathology 98:S117

Taro (*Colocasia esculenta*) germplasm from Thailand, Vietnam, and Nepal as well as 11 hybrids derived from Hawaiian, Thai, Samoan, Guamanian, Papua New Guinean, Palauan, and Indonesian parents were screened for resistance to *Meloidogyne javanica* in the greenhouse. All accessions and hybrids supported nematode reproduction. Overall, the hybrids tended to grow more and support less nematode reproduction than the taro cultivars from Thailand, Vietnam, and Nepal. Accession 035 from Thailand and Hybrid 40 had consistently lower Reproductive factors (Rf) and higher growth rankings than the other taros suggesting possible resistance. Hybrid 19 had superior growth (166.2 g) compared to the overall mean of the other hybrids and supported high levels of nematode reproduction, suggesting tolerance to the nematode. Complete host-plant resistance to *M. javanica* remains elusive in taro.

Prophylactic foliar fungicide and insecticide applications and their impact on soybean yield components

L. M. ORTIZ-RIBBING (1), G. K. Roskamp (2), M. D. Roegge (1)
(1) University of Illinois Extension; (2) Western Illinois University
Phytopathology 98:S117

Current soybean prices have encouraged the interest and continued use of prophylactic fungicides and insecticides applications. Applying preventative fungicide and insecticide treatments for plant health and their impact on soybean yield and yield components was investigated, for a second year, at two locations (Macomb and Perry) in Western Illinois. Pesticide applications [azoxystrobin (Quadris), pyraclostrobin (Headline), and lambda-cyhalothrin (Warrior)] to early (R3) and late (R2) maturing soybean cultivars occurred in July 2007 with insect populations and disease ratings taken at 0, 10 and 20 days. Yield components (soybean pods per plant, unfilled pods per plant, seed number per plant, seed weight per plant, and 100 seed weight per plot) were measured on ten plants from the center rows of four replicate plots. Both locations had low insect and disease pressure across plots. Yield components were not affected by fungicide or insecticide applications at Macomb; however, total yield was higher in Headline + Warrior compared to the

unsprayed control. At Perry, 100-seed-weights were higher in Headline + Warrior compared to Warrior alone, but total yield was not different. Soybean cultivar had an effect on both yield components and total yields. Total yield and 100-seed-weights were higher in the late maturing cultivar at Macomb, and pods per plant, seed number per plant, and seed weight per plant were higher in the late maturing cultivar at Perry. Seed number per plant was higher in the early maturing cultivar at Perry. Seed weight and number of pods per plant were correlated ($R^2 = 0.34$) with yield at Macomb, and number of seeds per plant and seed weight were correlated with yield ($R^2 = 0.25$) at Perry. Results reinforce the uncertainty of prophylactic fungicides and insecticides treatments and suggest that soybean response may vary by cultivar and maturity group.

Disease impacts in red pine managed to increase stand complexity
M. E. OSTRY (1), M. Moore (1)
(1) USDA Forest Service, St. Paul, MN, USA
Phytopathology 98:S118

Red pine (*Pinus resinosa*) is the most widely planted species in Minnesota. Research is underway to diversify the age and composition structure of stands to create two-cohort, mixed species stands that resemble the historical complexity of red pine ecosystems. A replicated study was established on four 64 ha sites in 80 year-old red pine stands. In 2002 three thinning treatments created 0.3 and 0.1 ha gaps which have aggregated retained trees into large and small groups and a traditional shelterwood resulted in trees retained evenly. No harvest control treatments were included on each site. Red, white (*P. strobus*) and jack (*P. banksiana*) pine seedlings were planted into each of four 16 ha treatment blocks on each site. Pre- and post-treatment data on disease incidence and severity have been collected on overstory and understory trees. Over 75 *Armillaria ostoyae* root rot centers ranging in size from 6-40 m in diameter were mapped before harvest. Several centers were inadvertently enlarged during thinning to create the canopy gaps. Many seedlings of all three species planted into these centers are dying from root rot. A high proportion of planted red and jack pine are affected by shoot blights caused by *Diplodia* spp. and *Sirococcus conigenus*. Jack pine seedlings are being killed by multiple main-stem galls caused by *Cronartium quercuum*. Both naturally seeded and planted pine seedlings are being impacted by multiple pathogens that may threaten management objectives. The canopy gaps resulting from *Armillaria* root rot, however, may diversify these stands as evidenced by several regenerating hardwood tree and shrub species within the centers that are not being damaged by the pathogen.

Death and recovery of fire damaged fine roots in a 35-year-old longleaf pine stand
W. J. OTROSINA (1), C. H. Walkinshaw (1)
(1) USDA Forest Service, Athens, GA
Phytopathology 98:S118

We employed histological analyses to study fine roots of longleaf pine trees after applying three controlled burn treatment intensities: hot, medium, cool and an unburned check. Studied stands were located at the Savannah River Site near New Ellenton, SC. Fine roots in mineral soil to a depth of six cm and fine roots within the humus layer were sampled, fixed, sectioned, and examined two, four and six months post treatment. Of roots in mineral soil, the hot burn treatment had the highest proportion of dead roots (0.375) six months post treatment versus 0.225 for roots from unburned check trees. The trees in the cool, medium, and unburned check tended to have fine root mortality amounts that trend lower after presenting initially high proportions of dead roots; ranging from 0.434 – 0.466 at two months to the 0.010 – 0.225 range after six months. We also enumerated starch grains within fine roots from mineral soil. Over the three sample periods, all burn treatments had less average starch grain counts per cell compared to those from unburned checks (treatment – checks = contrast), with the contrast being highest in the six month sample from the hot burn treatment (contrast = –2.75) followed by the medium and cool burn intensities (-1.89 and – 0.90, respectively). Fine root mortality proportions in the humus layer remained high during the two, four, and six month sampling in the hot burn treatment (0.63, 0.46, and 0.57, respectively). The unburned check root mortality proportions in this layer at two, four, and six months were 0.46, 0.28, and 0.25, respectively. These data suggest recovery of fine roots are retarded following hotter fires and may contribute to the reported post-fire decline in these stands, which had not undergone prescribed burning for the previous seven years.

Improvement in screening for resistance to *Sclerotinia sclerotiorum* in common bean through characterization of the pathogen and utilization of multi-state nurseries
L. K. OTTO-HANSON (1), J. Steadman (2)
(1) University of Minnesota-St. Paul, USA; (2) University of Nebraska-Lincoln, USA
Phytopathology 98:S118

Multiple sites in major production areas were used to validate putative new sources of resistance in common bean, *Phaseolus vulgaris*. Experiments were conducted to test variation of *Sclerotinia sclerotiorum* isolates used to screen for resistance in both the greenhouse and field plots in bean production areas. These tests were mycelial compatibility grouping (MCGs) for clonality and pathogen aggressiveness evaluation. Sixty-five MCGs were found; 37 of the MCGs were composed of a single isolate. Six MCGs were sampled frequently over multiple screening locations. Differences in isolate aggressiveness could be detected using the straw test. Differences in aggressiveness were found between isolate MCGs, however no differences in isolate aggressiveness were found among isolates within the same mycelial compatibility grouping. This data supports the hypothesis that isolate aggressiveness is associated with MCGs. The pathogen variation found among isolates could affect interpretation of results from multi-site screening nurseries. Isolates were also collected from three producer fields from each of four major bean production regions in the U.S. Producer field isolates were characterized for MCGs and tests of aggressiveness. The hypothesis that isolates found in natural populations in producer fields were similar to those identified in the plots used to screen bean lines and potential cultivars for white mold resistance. If more pathogen variation is found in screening plots than producer fields, the screening system may need to be re-evaluated.

Effectiveness of extended duration row covers for suppression of bacterial wilt in muskmelon
A. OWENS (1), L. Jesse (1), M. L. Gleason (1), J. Batzer (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 98:S118

A 2007 field trial in Gilbert, Iowa assessed effectiveness of extended-duration row covers for suppression of bacterial wilt (pathogen: *Erwinia tracheiphila*) on muskmelon. Experimental design was a randomized complete block with four treatments and four replications. Subplots were 30-ft-long single rows of cv. Athena with black plastic mulch, drip irrigation, and 2-ft. transplant spacing. Treatments were: 1) no row cover; 2) row covers removed at anthesis; 3) row covers removed 10 days after anthesis, with row cover ends opened at anthesis to allow for pollination; and 4) row covers removed 10 days after anthesis, with bumble bee boxes (Koppert Inc.) inserted under row covers at anthesis to provide pollination. After row cover removal, plants were assessed weekly for wilt symptoms. At harvest, marketable melons were counted and weighed. Early in the season, all row cover treatments had significantly lower incidence of wilt than the non-covered control, in which incidence increased to more than 75% by first harvest. For the treatment with row cover removal at anthesis, bacterial wilt incidence increased gradually to nearly 50% by harvest, whereas incidence remained below 10% for the extended-duration row cover treatments. Row cover treatments had higher marketable yield than the non-covered control, and yield was highest in the extended-duration row cover treatments. Although marketable yield did not differ significantly between the extended-duration treatments, the addition of bumble bees increased earliness by about 1 week.

Effect of conidial seed treatment rate of entomopathogenic *Beauveria bassiana* 11-98 on endophytic colonization of tomato seedlings and control of Rhizoctonia disease
B. H. OWNLEY (1), M. M. Dee (1), K. D. Gwinn (1)
(1) The University of Tennessee
Phytopathology 98:S118

Seed application of *Beauveria bassiana* isolate 11-98 can protect tomato seedlings from damping off caused by *Rhizoctonia solani*. The objectives of this study were to determine the effect of rate of conidia of *B. bassiana* applied to seed on disease control, and on extent of endophytic colonization by *B. bassiana* of seedlings. Conidia of *B. bassiana* were applied to seed in 2% methylcellulose then air-dried. Conidial rates were log 2, log 3, log 4, log 5, log 6, and log 7 conidia per seed. Two controls, seed treated with only 2% methylcellulose, and untreated seed were prepared also. The experiment was designed as a RCB with eight treatments and 15 replicates, with two seedlings per replicate. All seeds were planted into soil mix infested with *R. solani*. In a separate experiment, these conidial rates were applied to seed under gnotobiotic conditions to determine the extent of endophytic colonization in 3-week old seedlings. The degree of disease control achieved was dependent on initial population density of *B. bassiana* conidia on seed. Seedling height was significantly greater and disease ratings were significantly lower for plants grown from seed treated with log 6 or log 7 conidia per seed compared to the controls and other seed treatment rates. Using standard plating techniques onto selective medium, endophytic *B. bassiana* 11-98 was recovered from surface-sterilized roots, stems, cotyledons, and leaves of tomato seedlings grown from conidia-treated seed. As the rate of conidia applied to seed increased, the proportion of plant tissues from which *B. bassiana* 11-98 was recovered increased. Endophytic colonization was more extensive and consistent in plants grown from seed treated with log 7 conidia per seed.

Virus-induced gene silencing of soybean rust resistance genes in *Glycine tomentella*

A. PADMANABAN (2), S. Chang (1), S. Rosales-Mendoza (1), G. L. Hartman (1), S. S. Korban (1), S. Ghabrial (2)
(1) University of Illinois, Urbana, IL 61801; (2) University of Kentucky, Lexington, KY 40546
Phytopathology 98:S119

Soybean rust, incited by the fungal pathogen *Phakopsora pachyrhizi*, is a serious foliar soybean disease capable of causing major economic yield loss. Specific resistance to *P. pachyrhizi* is known and single dominant genes have been identified in soybean ($Rpp_{1\text{-}4}$), but these genes have been deemed ineffective to many isolates of *P. pachyrhizi*. It is therefore essential to search for other sources of stable genetic resistance, particularly those found in *Glycine* germplasm accessions. Such resistance genes have been identified in the wild perennial relative *G. tomentella*. Genes involved in rust resistance in other plant species including wheat, barley, rice, and maize have been reported to have homology to receptor-like kinase (*RLK*) genes. In soybean, several *RLK* genes have been identified, among which the leucine-rich repeat (LRR) RLKs are believed to be important in plant disease resistance. In this study, we utilized a virus-induced gene silencing (VIGS) approach to evaluate the contribution of three soybean *RLK* genes (*GmRLK1*, *Gm RLK2*, and *GmRLK3*), which are similar in amino acid sequences, to rust resistance. We employed the novel bean pod mottle virus (BPMV)-based VIGS vector to effectively silence the RLK genes in both soybean and *G. tomentella*. Recombinant BPMV vectors containing a 300-bp fragment from *GmRLK1* and a 258-bp fragment from *GmRLK3* were constructed and used to inoculate rust-resistant *G. tomentella* accessions. Results showed that silencing of *G. tomentella* homologs of *GmRLK* genes suppressed resistance to rust. A rating of 1 was scored for the vector control (no lesions), while ratings of either 4 or 5 (moderate or heavy lesion numbers, respectively) were recorded for *RLK*-silenced accessions. We are currently using the BPMV-VIGS vector to screen additional candidate rust resistance genes in *G. tomentella*.

A new and distinct species in the genus *Caulimovirus* exists as an endogenous plant pararetroviral sequence in its host, *Dahlia variabilis*

V. Pahalawatta (1), K. Druffel (1), H. PAPPU (1)
(1) Washington State University, Pullman, WA, USA
Phytopathology 98:S119

Viruses in certain genera in the family *Caulimoviridae* were shown to integrate their genomic sequences into their host genomes and exist as endogenous pararetroviral sequences (EPRV). However, members of the genus *Caulimovirus* remained to be the exception and are known to exist only as episomal elements in the infected cell. Dahlia mosaic caulimovirus (DMV) is an important viral disease of dahlia (*Dahlia variabilis*). Our surveys of dahlias to determine the incidence of DMV revealed the overwhelming presence of another distinct caulimovirus, designated DMV-D10, and 98% of the samples tested were positive for DMV-D10 irrespective of the symptom expression. Moreover, our studies have also shown that DMV-D10 is transmitted through seed to 100% of the progeny plants resulting in establishment of infection and symptom expression. These results indicated the possibility of integration of DMV-D10 viral sequences into the host plant genome. Evidence was obtained that the DNA genome of DMV-D10 is integrated into its host genome. Using cloned viral genes as probes, Southern blot hybridization of total plant DNA from dahlia seedlings showed the presence of viral DNA in the host DNA. Fluorescent in situ hybridization using labeled DNA probes from the D10 genome localized the viral sequences in dahlia chromosomes. The natural integration of a caulimovirus genome into its host and its existence as an EPRV suggests the co-evolution of this plant-virus pathosystem.

Characterization of *Aspergillus* section *Nigri* group-maize interactions by a green fluorescent protein-tagging approach

E. R. PALENCIA (1), A. E. Glenn (2), C. W. Bacon (2)
(1) Plant Pathology Department, University of Georgia, Athens, GA, USA; (2) USDA, ARS, Russell Research Center, Athens, GA, USA
Phytopathology 98:S119

Ochratoxin A, produced by some members of the *Aspergillus* section *Nigri* group, is a potent nephrotoxic and a potential carcinogenic mycotoxin. Some strains of *A. niger* and *A. carbonarius* are ochratoxin producers in plant substrates, including corn, coffee, grapes, onions, and peanut. However, there is little information on the nature of the interactions between these black-spored aspergilli and their plant hosts that lead to the accumulation of ochratoxin A is still unknown. Here we report the use of a green fluorescent protein (gfp) tagging approach to monitor the early interactions between *A. niger* and *A. carbonarius* gfp-expressing transgenic isolates and a maize line. Protoplasts from isolates were transformed by homologous conjugation, using a transformation vector pCT74 that contains SGFP from a ToxA promoter and a hygromycin gene as selective agent. The constitutitve gfp expression on transgenic isolates were monitoring by fluorescent microscopy. Our results showed that both *A. niger* and *A. carbonarius* can endophytically infect maize tissues.

Occurrence in the U.S. of tar spot, caused by *Diatractium cordianum*, on orange Geiger, *Cordia sebestena*

A. J. PALMATEER (1), J. M. Perez (1), R. A. Cating (1), R. C. Ploetz (1)
(1) University of Florida
Phytopathology 98:S119

Cordia sebestena is an attractive tree that produces small orange flowers in the spring and summer months. It tolerates sandy soils and sea spray, making it an ideal landscape tree for South Florida. In July 2007, a tar spot was observed on *C. sebestena* in the landscape and a commercial nursery in Homestead. Circular, hypertrophied spots were slightly chlorotic on the abaxial surface with numerous blackened stroma on the adaxial surface. Embedded in stroma were numerous circular to irregular perithecia, 173-312 µm in dia, with lateral necks up to 200 µm in length and 73-104 µm in dia. Asci, 77-92 X 11-13 µm, contained elongate, two-celled ascospores, 50-61 X 3-5 µm, with a conspicuous constriction at the dividing cell wall. These dimensions and the pathogen's morphology matched published descriptions for *Diatractium cordianum* (Ellis & Kelsey) Syd. DNA was extracted and a PCR was performed followed by sequencing of the ITS1, ITS2, and 5.8s rDNA. Ascospores were harvested from affected leaves and sprayed, at a concentration of approximately 104 ml^{-1}, on detached, symptomless leaves of *C. sebestena*. Inoculated leaves were placed on moist paper towels in Petri plates and maintained in a growth chamber at 25°C with fluorescent light at 10 h day^{-1}. Symptoms began to develop after 21 days and perithecia of *D. cordianum* were evident after 28 days. This is a new host record for *D. cordianum*, and is the first time the pathogen has been reported in the U.S.

Evaluation of heated potassium sorbate solutions to control postharvest green and blue molds on commercially important citrus cultivars

L. PALOU (1), C. Montesinos-Herrero (1), C. Pastor (1), M. del Río (1)
(1) IVIA, Montcada, València, Spain
Phytopathology 98:S119

Alternatives to conventional chemical fungicides to control citrus green and blue molds are needed to reduce health and environmental risks. Treatments for 2–3 min with 2–3% potassium sorbate (PS) solutions heated to 40–50°C have shown value to reduce these diseases. However, for commercial reasons, shorter treatments are desirable. Several commercially important citrus species and cultivars were artificially inoculated with *Penicillium digitatum* or *P. italicum* (10^6 spores/ml), immersed 24 h later for 5, 15, 30, or 60 s in water (control) or aqueous solutions of 3% (wt/vol) PS at 20, 53, 58, 62, 65, or 68°C, depending on the experiment, rinsed with tap water, and incubated at 20°C for 7 days. The most effective treatments were PS applications at 62°C for 30 or 60 s, which reduced green and blue molds by up to 20, 25, 50, 80, or 95% on 'Clemenules' and 'Nadorcott' mandarins, 'Fino' lemons, 'Ortanique' mandarins, and 'Valencia' oranges, respectively. After 60 days storage at 5°C, green mold and blue mold on 'Valencia' oranges treated with PS at 62°C for 60 s were reduced by 96 and 83%, respectively. Treatments applied to unwounded fruit before inoculation did not induce disease resistance. The use of brief dips at higher temperatures may increase the suitability of PS treatments as an alternative commercial control mean.

Navel orangeworm (*Amyelois transitella*) as a vector of *Aspergillus flavus* on almonds

J. D. PALUMBO (1), N. E. Mahoney (1), D. M. Light (1)
(1) USDA, ARS, Albany, CA, USA
Phytopathology 98:S119

Aflatoxin contamination of almonds results from infection by *Aspergillus flavus*. Damage of almond fruits by insects, particularly by navel orangeworm (NOW), is correlated with increased incidence and extent of aflatoxin contamination. However, no studies have shown a causal relationship between almond feeding by NOW and *A. flavus* infection. To demonstrate that NOW could transport and deposit *A. flavus* spores, NOW eggs were dusted with *A. flavus* spores and placed into open-bottom microfuge tubes on the surface of potato dextrose agar plates. Within 24 hours following egg hatch, NOW larvae were observed exiting the tubes, and *A. flavus* colonies developed along trails left by the larvae on the agar surface. Vector activity on almonds was assessed by incubating open bottom tubes containing *A. flavus*-dusted NOW eggs with individual almond kernels. After 21 days, NOW feeding damage and *A. flavus* colonies were observed on treated almonds, and not on almonds treated only with *A. flavus* spores. Aflatoxin levels in NOW-damaged, *A. flavus*-infected almonds were significantly higher than in control treatments, even in the absence of visible external *A. flavus* growth. These studies demonstrate that NOW is likely to act as a vector for *A. flavus* on almonds, and further emphasize the need for sorting to remove insect-damaged almonds to reduce the potential for aflatoxin contamination.

Detection of *Cucurbit yellow stunting disorder virus* (CYSDV) in cucurbit leaves using sap extracts and real-time, quantitative polymerase chain reaction (qPCR)

L. C. Papayiannis (1), S. C. Hunter (2), J. K. BROWN (2)
(1) Agricultural Research Institute, 1516 Nicosia, 22016 Cyprus; (2) Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA
Phytopathology 98:S120

During the last decade, *Cucurbit yellow stunting disorder virus* (CYSDV) (*Crinivirus*: *Closteroviridae*), native to the Middle East, has become a major constraint to cucurbit production, worldwide. Infected plants exhibit bright yellow, interveinal chlorosis on older leaves, yellow splotches on developing leaves, and overall stunting and reduced fruit quality. The host range of the virus includes cucumber, melons, squashes, and watermelon. The virus is transmitted by *Bemisia tabaci* (Hemiptera: Aleyrodidae) in a semi-persistent manner. The 2007 introduction of exotic CYSDV into Arizona and California, USA and in Sonora, Mexico mandates a regional management approach based on improved understanding of the epidemiology, including vector dynamics and virus-vector biology, and the identification of natural reservoirs. To aid in research toward effective disease management, a TaqMan® real time fluorescent, one-step reverse transcription (RT), quantitative polymerase chain reaction (PCR) assay has been developed and optimized. The assay is >100-fold more sensitive than conventional RT-PCR, and involves template preparation that does not require RNA purification. The assay can be accomplished either by first spotting the sap extract on a positively charged nylon membrane and elution, or by the direct addition of crude plant extract into the real time reaction cocktail. Preliminary results indicate that this real time method is capable of detecting CYSDV in cucurbit samples from the USA, Mexico, and the Mediterranean Basin, and provides the first simple, rapid and cost effective for a large number of samples.

New and emerging virus threats for nursery and ornamental crops in the Pacific Northwest

H. PAPPU (2), K. Eastwell (1), K. Druffel (2)
(1) Washington State University, IAREC, Prosser, WA, USA; (2) Washington State University, Pullman, WA, USA
Phytopathology 98:S120

The ornamental industry in the U.S. is valued at approximately U.S. $15 billion. A significant number of wholesale operations that produce and supply nursery/landscape and ornamentals plants are located in the western U.S., especially in California, Oregon and Washington. Recent surveys for viruses in nursery crops have shown the presence of several new and potentially important viruses. The following viruses were detected during surveys in Washington State: *Canna yellow mottle virus* (CaYMV) in canna (*Canna indica*), *Hosta virus X* on hosta (*Hosta* spp.), and *Tobacco rattle virus* in peony (*Paeonia* spp.). Increased awareness and avoiding the propagation and distribution of infected plants are necessary to minimize further spread of these viruses. A new carlavirus, *Helleborus net necrosis virus*, associated with the devastating 'black death disease' of hellebores (*Helleborus* spp.) was characterized. Enhanced efforts in controlling aphids has dramatically reduced the spread of this aphid-transmitted disease in nurseries and the development of reliable assay methods allowed nurseries to screen new additions to their breeding and propagation stock to exclude infected plants. Two tymoviruses have been identified in diascia (*Diascia* spp.) and nemesia (*Nemesia* spp.) ornamental plants: *Nemesia ring necrosis virus* and *Diascia yellow mottle virus* were found to be wide spread in many cultivars of these plants. RT-PCR-based assays for rapid and sensitive detection of these new and emerging viruses were developed and surveys are being carried out to determine the extent of their incidence and to develop virus-elimination strategies and production of virus-free stock.

Use of conserved genomic regions in a PCR-based assay for the detection of members of the genus *Caulimovirus*

H. PAPPU (1), K. Druffel (1)
(1) Washington State University, Pullman, WA, USA
Phytopathology 98:S120

Genus *Caulimovirus* (family *Caulimoviridae*) has several distinct species that include *Carnation etched ring virus*, *Cauliflower mosaic virus* (CaMV), *Dahlia mosaic virus* (DMV), *Figwort mosaic virus* (FMV), *Horseradish latent virus*, *Mirabilis mosaic virus*, *Strawberry vein banding virus* (SVBV), and *Thistle mottle virus*. Caulimoviruses are characterized by a double stranded DNA genome of ca. 8 kb in size and they infect a wide range of plant species and produce varying degrees of symptoms. A comparative analysis of the sequences of the known *Caulimovirus* species revealed two regions that are conserved in all caulimoviruses with the exception of SVBV. Degenerate primers based on these two regions were designed and tested in a PCR-based assay for broad spectrum detection of members of this genus. CaMV, FMV, and three distinct caulimoviruses associated with dahlia (*Dahlia variabilis*) were used to show the utility of this test in detecting diverse caulimoviruses. The primer pair gave an amplicon of expected size (840 bp). Amplicons from each virus were cloned and sequenced to verify their identity. The primer pair and the PCR assay provide a rapid approach for the broad spectrum detection of several viruses in the genus Caulimovirus.

Detection of *Ralstonia solanacearum* race 4 in field samples using a combination of serological and molecular assays

M. L. PARET (1), R. Kubota (1), D. M. Jenkins (1), A. M. Alvarez (1)
(1) University of Hawaii at Manoa
Phytopathology 98:S120

Ralstonia solanacearum race 4 (Rs) causes bacterial wilt of edible ginger (*Zingiber officinale*). Serological and DNA-based assays were evaluated for detecting low populations of Rs in soil and effluent water. Rs strain A4515 was poured over soils containing wounded and non-wounded gingers. Survival of Rs in effluent water and/or soil samples was analysed for 150–180 days after inoculation (DAI) by viable plate counts, ELISA, PCR and LAMP. Soil and effluent water samples also were analyzed by an Immunostrip assay and results were compared with viable plate counts. Recovery of viable Rs was 2 log units higher from soil samples than from effluent water collected from the same pot. ELISA using Ps1, an anti-EPS mAb, showed consistent positive results for effluent water with Rs populations above log 4.0 cfu/mL. An immunostrip assay based on Ps1a, a similar antibody, gave positive results for effluent water when culturing showed Rs populations of log 1 cfu/mL. Likewise, immunostrip results were positive in soil samples containing log 2 cfu/g. Both PCR and LAMP detected Rs in effluent water with populations above log 3 cfu/mL. Rs-EPS was detectable in effluent water till 130 DAI (log 1 cfu/mL) by immunostrips while ELISA, PCR and LAMP assays in effluent water samples detected Rs for only 50 DAI. The immunostrip assay is highly sensitive and can be effectively used in combination with other assays for assessing Rs in field samples.

Identification of pathogen-responsive proteins from soybean leaves during interaction of soybean and *Phakopsora pachyrhizi* using proteomics

S. PARK (1), Z. Chen (1), A. Chanda (1), N. Hazard (1)
(1) Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, USA
Phytopathology 98:S120

Asian soybean rust, caused by *Phakopsora pachyrhizi*, is an emerging disease in the Continental U.S. since its discovery in late 2004. The disease has spread rapidly in the past three years; with nineteen states reported soybean rust in 2007. This disease has the potential to cause severe yield reduction and billions of dollars in economic losses to U.S. soybean growers since all of the U.S. commercial soybean varieties are susceptible to this disease. In an effort to understand soybean and soybean rust interaction, we examined protein profile changes over a 14-day period in soybean leaves of one susceptible commercial line (Pioneer 95M960) with or without soybean rust inoculation. Proteins were extracted from the inoculated and control leaves collected at 10h, 1, 2, 3, 4, 6, 10, and 14 days after inoculation, and resolved on 2-DE gel. Five proteins, including a pathogenesis-related protein 10 (PR10) and chalcone-flavone isomerase (CHI), were significantly up-regulated from 1 to 6 days after inoculation. The induction can be observed as early as 10 hr after inoculation. This early induction of defense related genes indicate their importance in host defense against *P. pachyrhizi* infection. Currently, the levels of PR10 and CHI transcripts are being determined using quantitative real-time PCR (qRT-PCR). This information will help us to understand soybean and *P. pachyrhizi* interaction, and to develop new strategies to enhance soybean resistance to rust disease in the future.

Incidence and diversity of dsRNA in a Korean population of the chestnut blight fungus, *Cryphonectria parasitica*

S. PARK (4), J. Kim (4), H. Chung (4), J. Lim (1), B. Kwon (4), K. Lee (4), J. Kim (4), M. Kim (3), B. Cha (1), S. Lee (3), K. Kim (3), Y. Lee (2), M. Yang (4), D. Kim (4)
(1) Department of Agricultural Biology, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea; (2) Department of Herbal Medicine, Hoseo University, Asan, Chungnam 336-795, Korea; (3) Division of Forest Insect and Diseases, Korea Forest Research Institute, Seoul 130-712, Korea; (4) Institute of Molecular Biology and Genetics, Research Center of Bioactive Materials, Chonbuk National University, Jeonju, Cho
Phytopathology 98:S120

676 isolates from 33 Korean *Cryphonectria parasitica* subpopulations in Korea were analyzed for dsRNA incidence and diversity. DsRNA was detected in 84 isolates. Although the dsRNA band patterns varied in several minor bands, infected isolates could be categorized into two groups. The most common band pattern occurred in 77 isolates, and contains a 12.7 kb band which is indicative of *Cryphonectria hypovirus* 1 (CHV1), and several accompanying minor bands with sizes ranging from 5.0-0.9 kb. Northern blot analysis revealed that all the 12.7 kb fragments in the dsRNA-containing

isolates hybridized to probes corresponding to open reading frames (Orf) A and B from reference CHV1 (GenBank Accession No. M57938). In addition, the sequence 1.4 kb of a cDNA fragment from a representative isolate of the most common group showed 99% sequence similarity to OrfA of CHV1. The other group of seven isolates, however, evidenced distinctive bands of 3.5 kb and 3.3 kb, without the 12.7 kb band. Sequence comparison of cloned fragments of these dsRNAs was similar to those of the coat protein and RNA dependent RNA polymerase genes of Chrysovirus, which indicates the occurrence of Chrysovirus in the Korean population. Fungal strain identity was assessed via restriction fragment length polymorphism (RFLP) of the internal transcribed spacers (ITS). Among the 84 tested isolates, six evidenced different ITS-RFLP patterns (RFLP-II) from that (RFLP-I) of *C. parasitica*, and were believed to be *C. nitschkei*, a sympatric species reported on chestnut trees in Japan. The chryosvirus and CHV1, were detected on strains showing both RFLP patterns. However, the chrysovirus was more frequent in the RFLP-II group.

Penetration process of pathogen and comparison of stomatal structure between susceptible and resistant varieties to grapevine leaf spot

J. PARK (1), K. Han (1), Y. Han (1), S. Lee (1), S. Cheong (1)
(1) Horticultural Environment Div., National Horticultural Research Institute, RDA, Suwon 441-440, Korea
Phytopathology 98:S121

Grapevine leaf spot caused by *Pseudocerspora vitis* is one of the most important diseases in Korea. However, very few papers were reported throughout the world. Therefore, these studies were carried out to know pathogenicity of the pathogen isolated in several location of Korea and penetration mechanism of the pathogen. Inoculation tests at room temperature were applied on both sides of leaves with different isolates of the fungus. The typical symptoms were appeared in the abaxial surface leaf inoculation, but no symptoms appeared at all in adaxial surface leaf inoculation with all isolates. The average incubation period was nine days because all symptoms appeared from 8 to 10 days after inoculation. In order to know the mechanism of invasion of *P. vitis* to grapevine, two cultivars having different degree of resistance were compared by observing the behavior of penetration hyphae through stomata. In susceptible cultivar "Campbell Early", the fungus penetrated readily into stomata after inoculation. However, in resistant cultivar "Kyoho" the fungus seemed not recognize the site of stomata and pass over or surrounded only the guard cell. Therefore, the fungus was not able to penetrate the resistant cultivar "Kyoho". In comparison of height of guard cells of stomata between susceptible and resistant cultivars, significant differences were observed by scanning electron microscope and light microscope. The height of guard cells of "Campbell Early" showed a little higher than those of "Kyoho" known to be resistant cultivar to the fungus.

Viability of *Phytophthora ramorum* after passage through slugs

J. L. PARKE (1), A. Oguchi (2), E. J. Fichtner (2), D. M. Rizzo (2)
(1) Oregon State University, Corvallis, OR; (2) University of California-Davis, Davis CA
Phytopathology 98:S121

Phytophthora ramorum, the causal agent of sudden oak death, produces abundant chlamydospores; however, understanding the role of these potential survival structures in the disease cycle is difficult due to the low and variable rate of chlamydospore germination. Oospore germination of other *Phytophthora* spp. may be stimulated by passage through the alimentary canal of snails, but the effects of chlamydospore ingestion by molluscs are unknown. The viability of *P. ramorum* colonies was investigated after passing them through the alimentary canal of banana slugs (*Ariolimax* spp.) and grey garden slugs (*Derocerus reticulatum*). Slugs that were fed V8 agar cultures of *P. ramorum* produced feces that contained hyphae and chlamydospores. Broth-grown hyphae and chlamydospores were also applied to strawberries and fed to slugs. Slug feces from both sources plated onto *Phytophthora* selective media yielded *P. ramorum* colonies. Microscopic observation showed that many chlamydospores in fecal samples either germinated directly, often with multiple germ tubes, or indirectly to form sporangiophores. Sporangia production was abundant on fecal surfaces. Tanoak leaves inoculated with feces from culture-fed slugs became infected by *P. ramorum.* These results suggest that a portion of chlamydospores and hyphae that pass through the alimentary canal of slugs remain viable. The potential effect of slugs on chlamydospore germination and the possible role of slugs in disease transmission will be investigated.

A systems approach for managing *Phytophthora* diseases in production nurseries

J. L. PARKE (1), N. Grunwald (2), C. Lewis (1), V. Fieland (2)
(1) Oregon State University, Corvallis, OR; (2) USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR
Phytopathology 98:S121

Nursery plants are susceptible to several diseases caused by *Phytophthora* species. Nursery plants are also important long-distance vectors of non-indigenous pathogens such as *P. ramorum*. Pre-shipment inspections have not been adequate to ensure that shipped plants are free from *Phytophthora*, nor has this method informed growers about sources of contamination in their nurseries. We applied a new approach based on Hazard Analysis of Critical Control Points (HACCP) for systematically detecting sources of *Phytophthora* contamination in four Oregon nurseries. We identified critical control points (CCPs) in commercial production systems and sampled bimonthly over a 15-month period. Plants, potting media, containers, irrigation water, and can yard substrates were sampled at all stages of production. Putative *Phytophthora* isolates were tested with genus-specific PCR and identified to species by direct sequencing of the internal transcribed spacer (ITS) rDNA. The most frequently encountered species were *P. cinnamomi*, *P. syringae*, *P. citricola*, *P. cryptogea*, *P. gonopodyides* and *P. citrophthora*. Results showed that healthy container plants often became contaminated when set out on contaminated can yard substrates. Used containers were sources of contamination at all four nurseries, as was water from irrigation ponds at two nurseries. After identifying CCPs where contamination occurred, we worked with nursery managers to develop best management practices (BMPs) specific for each nursery. Sampling will continue after BMPs are implemented to determine if this approach is successful in reducing *Phytophthora* contamination.

Dispersal of *Xanthomonas citri* subsp. *citri* bacteria downwind from harvested, infected fruit

P. E. PARKER (1), C. H. Bock (3), A. Z. Cook (1), T. R. Gottwald (2)
(1) USDA-APHIS-PPQ, Edinburg, TX, USA; (2) USDA-ARS-USHRL, Ft. Pierce, FL, USA; (3) USDA-ARS/University of Florida, Ft. Pierce, FL, USA
Phytopathology 98:S121

Citrus canker (*Xanthomonas citri* subsp. *citri*, *Xcc*) is a bacterial disease that severely damages citrus crops. Its recent introduction to Florida has created difficulties with international and domestic trade and movement of citrus material. This study examined the potential dispersal of bacteria from ripe, cankered fruit. Cankered, field collected fruit were placed in a cull pile or suspended in front of a wind/rain source. Wind speeds of 0, 10, and 25 m/sec were generated by an air boat fan which blew simulated rain from spray nozzles across the cankered fruit. The wind blown splash was collected on PVC panels and on young grapefruit trap plants in flush, positioned at 0, 2, 5, and 10 m from the fruit. The plants were incubated in a greenhouse and assessed for infection after 3 weeks. Only one plant downwind became infected, and developed a single lesion. Wind-blown spray collected on panels plated onto nutrient agar collected up to 470 total bacteria/ml. Typical xanthomonad-like yellow colonies accounted for up to 300/ml, and ELISA dot blot indicated up to 40/ml were possibly *Xcc*. Dispersal of *Xcc* from cull piles and suspended fruit appears to be low. Leaf infections rarely occur, even at high wind speeds.

The effects of grapevine rootstock cultivar and crop phenology on the culturable bacteria community of rhizosphere soil and endo-rhizosphere in a California vineyard

S. R. PARKER (1), D. A. Kluepfel (1)
(1) USDA, ARS, Crops Pathology Genetics Research Unit, Davis, CA, USA
Phytopathology 98:S121

The relationship between members of the soil-borne microbial community and some annual plant hosts are becoming more understood, but little is know about many significant perennial agricultural crops such as grapes. As in many other perennial production systems, modern viticulturalists generally produce wine grapes from vines grafted onto rootstocks. Modern grapevine rootstocks of *Vitis* and *Muscadina* parentage are used to provide vines with varying degrees of protection from soil problems including nematodes, lime, phylloxera, and drought. Developing an understanding of the grapevine rhizosphere bacterial community is needed to provide a baseline of information for further research concerning microbial diversity, disease control, and growth promotion. Five popular grapevine rootstocks, Teleki 5C, 110R, 101-14, Ramsey, and Saint George, were selected based on their parentage, and sampled over a production season. When quantifying the culturable population, a significant interaction was found between the rootstock cultivar and sample time ($P < 0.001$). Significant differences were found among the rootstock cultivars in August and November, but not in June. Richter 110R and Ramsey had lower populations of culturable rhizosphere bacteria in August, and Teleki 5C and 101-14 had higher populations in November. In assessing the carbon utilization profiles, a three way interaction between the cultivar, carbon source, and sampling time was discovered further illustrating differences in the bacterial communities as a function of host genotype. Identified bacteria members from rhizosphere soil and the endo-rhizosphere will be reported.

Temporal and spatial effects of long-term floor management on the bacterial and nematode communities in a Salinas Valley, California grape vineyard
S. R. PARKER (1), D. A. Kluepfel (1)
(1) USDA, ARS, Crops Pathology Genetics Research Unit, Davis, CA, USA
Phytopathology 98:S122

California grape producers traditionally manage vineyard floor vegetation to control weeds, vine vigor, and prevent erosion. However, little is known about how these vineyard floor management strategies impact the soil-borne microbial communities. In the final year of a long term vineyard floor management field study, we examined both the bacterial and nematode communities present in the row middles and berms across six different floor management strategies. Samples were assayed for total bacterial and nematode populations in addition to identifying individual members of these communities. An analysis of the culturable bacteria community found weed management and cover cropping practices had no significant quantitative effect on bacterial populations. Qualitatively, however, bacterial populations were altered as a function of cover crop and weed management practices. The grapevine rhizosphere bacterial populations were greater than populations in the row middles bulk soil during the grape root flush in the spring. During harvest and dormancy, bulk soil bacterial populations were greater in the rows than on the berm ($P < 0.05$). The nematode community in the row middles was significantly affected by both weed management and cover crop practices. In the berm, nematodes were not affected quantitatively or qualitatively under the six management regimes. Moreover, the nematode community of the berm was different from that found in the row. These row middle management strategies do not influence the bacterial and nematode communities in the berm where roots of the vine are concentrated.

Evolutionary and molecular population genetic analysis of *Impatiens necrotic spot virus* (INSV)
E. J. PARKS (1), J. Speck (1), J. Abad (2), I. Carbone (1), J. W. Moyer (1)
(1) North Carolina State University, Raleigh, NC, USA; (2) USDA-APHIS-PPQ-PHP-PSPI, Beltsville, MD, USA
Phytopathology 98:S122

Impatiens necrotic spot virus (INSV) is a highly destructive plant virus in the family *Bunyaviridae* that causes widespread and devastating losses in ornamental greenhouse crops. Our goal is to formulate an attribution system based on an understanding of the population genetics of INSV, similar to that for *Tomato spotted wilt virus* (TSWV). INSV exhibits geographical subdivision despite the shipment of plant material worldwide; it does not, however, have the extensive geographic structure of TSWV. Parsimony analysis does not illustrate the relationships between the isolates in a clear way as it does for TSWV due to unresolved polytomies and discordant phylogenies from the different genes. We present here an expansion of previous methods that builds on parsimony analysis, merges the data from the three genes, and improves the resolution of the phylogeny. Thirty-two isolates from six countries and seven U.S. states were selected for analysis based on the availability of sequence data for the N, NSm, and RdRp genes. Multiple isolates from North Carolina, California, and New Zealand permitted significance testing of population genetic statistics. Compatibility analysis was used to locate informative regions of the INSV genome and eliminate areas with phylogenetic conflict. The selected regions were examined for signatures of migration, population growth, and selection. Migration analysis suggested a recent divergence of the populations and no gene flow between populations, with sharing of ancestral polymorphisms the highest between the NC and CA populations. Neutrality testing revealed that the NZ population was evolving neutrally at all loci, but the evolutionary signatures of the loci in the NC and CA populations varied; some loci were evolving neutrally, while others contained evidence of population growth or background selection. This analysis of the INSV genome approximates a species tree for INSV and elucidates genomic regions with more useful phylogenetic information.

Population structure of wheat powdery mildew in the eastern U.S.
R. PARKS (3), I. Carbone (2), J. Murphy (1), C. Cowger (3)
(1) North Carolina State University Crop Science, Raleigh, NC, USA; (2) North Carolina State University Plant Pathology, Raleigh, NC, USA; (3) USDA-ARS, North Carolina State University Plant Pathology, Raleigh, NC, USA
Phytopathology 98:S122

In the eastern U.S., and other wheat growing areas of the world, powdery mildew (*Blumeria graminis* f. sp. *tritici*) is a major constraint to the production of wheat. The most effective and economical means of control is cultivar resistance, but high evolutionary potential enables the pathogen to overcome new resistance genes, often within several seasons of commercial deployment. Several surveys to determine population virulence to specific resistance genes have been performed. Little is known about *B. graminis* population structure, and virulence frequency data are sometimes used to infer structure. However, such inferences may be biased by local commercial cultivar choices. We developed a population of 141 single-ascospore derived powdery mildew isolates from susceptible cultivars in 10 locations in the eastern U.S. Virulence phenotypes of all isolates were determined on a set of 16 single-gene wheat differentials. From *B. graminis* housekeeping and repetitive DNA sequences, we developed primers for analysis of polymorphism distribution and population structure. Dendrograms based on Nei's (1972) standard genetic distance (G_{st}) inferred from multilocus virulence phenotypes indicated clustering of isolates into northern and southern subpopulations, with North Carolina as the boundary. DNA sequence based AMOVA analysis using pre-defined groups derived from Hudson's (2000) sequence based subdivision test (S_{nn}) supported this conclusion. Phylogenetic analysis was also performed. However, extensive homoplasy was detected, confounding phylogenetic inferences. A likely cause is sexual reproduction and recombination between genomic regions.

Variations in induced resistance response among cultivated tobacco types
V. PARKUNAN (2), C. S. Johnson (2), J. D. Eisenback (1), S. A. Tolin (1), J. Pattison (2)
(1) Virginia Tech Plant Pathology, Physiology, and Weed Science, Blacksburg, VA, USA; (2) Virginia Tech, Southern Piedmont AREC, Blackstone, VA, USA
Phytopathology 98:S122

Systemic acquired resistance (SAR) and induced systemic resistance (ISR) were compared among flue-cured (cv. K326, NC71, NC297), burley (cv. TN90), and oriental (cv. Xanthi) tobacco types. Bottom, middle, and top leaves were detached from 4 week old plants of TN90, NC297 and Xanthi at 3-day intervals following acibenzolar-*S*-methyl (ASM) treatment, and inoculated with *Tobacco mosaic virus*. Necrotic local lesion numbers on ASM-treated vs. untreated tobaccos differed by type, leaf position, and time after treatment. Lesion number decrease after treatment, an indicator of SAR, occurred by day 3 in TN90 and NC297 and by day 6 in Xanthi. SAR (ASM) and ISR (*Bacillus* spp.) were also compared between Xanthi and K326 by challenge with 5,000 eggs of tobacco cyst nematode (*Globodera tabacum solanacearum-GTS*) 5 weeks after seeding. Cysts were extracted 11 weeks later. Use of *B. subtilis* A13 + *B. pumilis* INR7 reduced *GTS* reproduction on K326, but not on Xanthi. However, both ASM and *B. subtilis* A13 + *B. amyloliquifaciens* IN937a (BioYield™) reduced GTS reproduction similarly on K326 and Xanthi. When Xanthi seedlings had been grown in a BioYield™-treated soil-less medium, a single ASM application at 200 ppm 1 week after transplanting significantly reduced *GTS* reproduction under field conditions. ASM and BioYield™ treatments reduced nematode development more consistently on *GTS* susceptible K326 than on resistant NC71.

Transcriptome of *Fusarium graminearum* during plant infection and toxin biosynthesis
M. Pasquali (2), E. Lysoe (1), K. Seong (2), J. Menke (3), H. KISTLER (2)
(1) Bioforsk, As, Norway; (2) USDA ARS Cereal Disease Laboratory, St. Paul, MN, USA; (3) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S122

To understand trichothecene accumulation and the infection cycle of the head blight pathogen *Fusarium graminearum*, fungal gene expression profiles were monitored during plant infection. Strains containing mutations in genes for three transcription factors were found to control trichothecene accumulation in planta and pathogenicity. Expression profiles were compared between wildtype and these mutants during infection of wheat. Mutants deleted for the *StuA* gene were greatly decreased in sporulation and produced no perithecia in culture. Unlike Delta*stuA* mutants in *F. oxysporum*, *F. graminearum* Delta*stuA* mutants were greatly reduced in pathogenicity. Reduced pathogenicity may be due to decreased trichothecene levels in planta, which in the mutant were <1% the levels of wildtype. Levels of transcripts corresponding to some genes involved trichothecene biosynthesis were greatly diminished in the Delta*stuA* mutant. Thus both sporulation and trichothecene synthesis may be regulated under the control of *StuA*. Mutants deleted for the transcription factors *TRI6* and *TRI10* also were diminished for both trichothecene accumulation and pathogenicity to wheat. Largely overlapping sets of approximately 200 genes were altered in expression $\geq$ two-fold in either Delta*tri6* or Delta*tri10* strains. In addition to genes responsible for trichothecene biosynthesis, genes involved in primary metabolism and transport also were significantly regulated by *TRI6* and *TRI10*. A model for global regulation and cross pathway control of sporulation, mycotoxin biosynthesis and pathogenicity will be presented.

Inheritance of soybean rust resistance in common bean
M. A. PASTOR-CORRALES (1), R. D. Frederick (2)
(1) ARS-USDA, Soybean Genomics and Improvement Laboratory, BARC-W., Beltsville, MD, USA; (2) ARS-USDA, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD, USA
Phytopathology 98:S122

The soybean rust (SBR) pathogen, *Phakopsora pachyrhizi*, has a very broad host range of leguminous crops including common bean, *Phaseolus vulgaris*

L. (dry and snap beans). SBR has been reported on common beans in South Africa, the United States, Argentina and Brazil. Among 16 common bean cultivars that were inoculated with six isolates of *P. pachyrhizi* from Asia, Africa and South America, the cultivar Compuesto Negro Chimaltenango (CNC) was the most resistant to all six isolates, while the cultivar Mexico 309 (Mx. 309) was among the susceptible. The inheritance of SBR resistance in CNC was studied by crossing the bean cultivars Mx. 309 (female) and CNC (male). F_1 seedlings were inoculated with an isolate of the dry bean rust pathogen (*Uromyces appendiculatus*) to insure that they were the product of a cross. The parents and ensuing F_2 seedlings were inoculated with the *P. pachyrhizi* isolate Brazil 01-1. Disease severity was evaluated using a 1–5 scale. A total of 140 F_2 plants were evaluated as resistant (severity 2 and 3) and 101 plants as susceptible (severity 4 and 5). The segregation for SBR resistance only fit a 9 resistant: 7 susceptible ratio with a Chi squared value of 0.26 ($P = 0.60$). These results support the hypothesis that SBR resistance in CNC is controlled by the interaction of two genes with complete dominance at both gene pairs; one dominant allele of each two genes is necessary to produce the resistant phenotype but either recessive homozygote is epistatic to the effects of the other gene.

Evaluation of sources of soybean rust resistance using detached leaves

C. PAUL (2), G. L. Hartman (1)
(1) USDA-ARS, Urbana, IL, USA; (2) University of Illinois, Urbana-Champaign, IL, USA
Phytopathology 98:S123

Soybean rust (SBR), caused by the fungus *Phakopsora pachyrhizi*, is a potentially devastating disease that can cause significant yield losses. Resistance in soybean germplasm, both qualitative and quantitative, may be effective in providing partial control of SBR. Thirty soybean accessions that contained a known major gene for resistance and/or were reported to have field resistance to SBR were evaluated for resistance to *P. pachyrhizi*. Detached leaves of the 30 accessions were inoculated with six U.S. domestic *P. pachyrhizi* isolates. Of the 30 accessions, two accessions had a hypersensitive reaction, eight had reddish brown (RB) lesions, and the remainder had tan lesions. There were significant differences by accession in the number of sporulating uredinia and the number of stained uredinia in fixed tissue. The number of sporulating uredinia and stained uredinia in RB lesions ranged from 0 to 7.7 with a mean of 0.67 per cm^2 and from 0 to 13.3 with the mean of 1.2 per cm^2, respectively. The number of sporulating uredinia and stained uredinia in tan lesions ranged from 2.3 to 56.3 with a mean of 26.2 per cm^2 and from 7.5 to 143 with the mean of 47.24 per cm^2, respectively. None of the resistance sources carrying the Rpp2 and Rpp4 genes were resistant to the six isolates. In conclusion, both qualitative and quantitative resistance was observed among these 30 soybean accessions to the six U.S. domestic *P. pachyrhizi* isolates.

Meta-analysis of hybrid corn yield response to foliar fungicides

P. A. PAUL (2), C. A. Bradley (3), A. E. Robertson (1), L. V. Madden (2)
(1) Iowa State University, Ames, IA 50011; (2) The Ohio State University/ OARDC, Wooster, OH 44691; (3) University of Illinois, Urbana, IL 61801
Phytopathology 98:S123

Recent claims of substantial yield increases in hybrid corn in response to foliar fungicides have led to millions of dollars being spent on fungicide application across the U.S. Corn Belt. However, these claims have generally not been substantiated by sound experiments and adequate data analyses. A random-effects meta-analysis was performed on yield data from replicated fungicide trials conducted in 10 U.S. states between 2004 and 2007 to determine the overall mean yield response to foliar fungicides. The fungicides tested were azoxystrobin, pyraclostrobin, azoxystrobin+propiconazole, and propiconazole+trifloxystrobin. For each trial, the difference in mean yield between fungicide-treated and untreated plots was used as the effect size and separate models were fitted to the data for each fungicide to determine the overall mean effect sizes. The difference in mean yield varied among trials and among fungicides, ranging from –30.9 to 52.0 bu/A for pyraclostrobin, with an overall mean of 3.8 bu/A; from –22.31 to 48.34 bu/A for azoxystrobin+propiconazole, with an overall mean of 3.8 bu/A; from –8.73 to 52.80 bu/A for azoxystrobin, with an overall mean of 5.5 bu/A; and from –28.55 to 47.78 bu/A for propiconazole+trifloxystrobin, with an overall mean of 4.7 bu/A. Subsequent analyses will be performed to evaluate the influence of moderator variables such as mean levels of disease, fungicide application timing, and hybrid disease resistance on the overall yield response due to each fungicide.

Quantification of *Pratylenchus penetrans* DNA in maize roots for greenhouse seed-treatment studies

C. PAVON (1), X. Gao (1), T. Niblack (1)
(1) University of Illinois, Urbana-Champaign, IL, USA
Phytopathology 98:S123

The use of seed treatments is being investigated for control of various plant-parasitic nematodes. The lesion nematode (*Pratylenchus penetrans*) penetrates plant roots, feeds on root tissue, and causes yield loss in a wide range of crops. We evaluated the efficacy of experimental seed treatments for control of lesion nematode on maize by quantifying nematode DNA in roots with real-time quantitative polymerase chain reaction (QPCR). A TaqMan assay was developed for the detection of the 18S ribosomal RNA gene of *P. penetrans*. In a greenhouse experiment, 15.2-cm-diam pots filled with a steam-pasteurized soil mixture (2 soil: 1 sand: 1 vermiculite) were infested with 500 *P. penetrans*; each pot contained one seedling. The experiment consisted of 22 treatments arranged in six randomized complete blocks. The experiment was harvested 45 days after planting and fresh root weights were determined; half of the root system was processed for lesion nematode counts and the other half for QPCR. Lesion nematodes were extracted from roots cut into 1 cm sections, placed in a 5% streptomycin sulfate solution, and shaken gently for 48 h. The roots used for QPCR were stored at –80°C and freeze-dried before DNA extraction. In comparison with other treatments, the check (without seed treatment) had higher lesion nematode counts and DNA quantities ($P < 0.01$). Moreover, the QPCR assay detected *P. penetrans* DNA in root samples where no lesion nematodes were extracted by means of the shaker technique. QPCR assay of nematode DNA in host roots is a sensitive and reliable indicator of seed treatment efficacy.

A PCR-based assay for detection of *Puccinia horiana* on chrysanthemums

K. F. PEDLEY (1)
(1) USDA Agricultural Research Service, FDWSRU, Ft. Detrick, MD 21702
Phytopathology 98:S123

Puccinia horiana (Henn.), the causal agent of chrysanthemum white rust (CWR), is an obligate biotrophic fungus that only grows and reproduces on twelve species of chrysanthemums. The disease is particularly problematic to commercial chrysanthemum propagation in greenhouses and nurseries where outbreaks can result in severe losses. CWR remained confined to Japan and China until 1963, but is now found in most countries where chrysanthemums are grown. Although CWR is not established in the United States, it has been introduced, detected, and eradicated in a number of locations. Infected plants produce pinkish-white or buff pustules (telia) on the lower leaf surface. Current identification protocols for CWR rely upon both macroscopic symptoms and microscopic examination of the teliospores, which are produced at a late stage of infection. For this reason, during the initial stages of infection the pathogen can often elude detection. The identification of plant pathogenic fungi based on molecular markers can be advantageous because such techniques do not require the presentation of fully developed morphological features, and typically can be used to confirm the presence of a pathogen at the earliest stages of an infection. Here I present the development of a PCR-based assay for the detection of *P. horiana*.

Effects of environmental conditions and sunflower growth stage on Phomopsis infection and severity

S. A. PEREYRA (1), A. Fassio (1), S. Stewart (1), M. Rodriguez (1)
(1) INIA - Natl Inst for Agric Research, Colonia, Uruguay
Phytopathology 98:S123

Phomopsis, caused by *Diaporthe helianthi*, has produced significant grain yield losses in Uruguay since 2002/2003. Experiments were conducted to evaluate the role of environmental variables and sunflower phenology on the onset and development of Phomopsis. Moderately resistant to susceptible sunflower genotypes with similar maturity were planted each week since mid-September in 15 and 20 dates in 2006/2007 and 2007/2008, respectively. Plots were managed under natural infection in 2006/2007 and inoculated with *D. helianthi*-infected residue in 2007/2008. Temperature, rainfall and relative humidity were hourly recorded. Growth stages were determined each week. Stem (SC) and head (HC) canker incidence and severity were assessed weekly since growth stage R6 and AUDPCs were calculated. The effect of planting date was significant for disease levels in both years; however disease onset was associated to favorable environmental conditions occurring since reproductive growth stages: mean relative humidity (RH) during 48–72 hours higher than 80% or the number of consecutive hours with RH higher than 80% greater than 20. Information from this study improved the accuracy of a warning system based on a French model for Uruguayan environment. HC were visible at 7–10 days after favorable conditions occurred, while SC were visible at 15–21 days. Data from this research indicate that planting date is not a useful disease management tool since optimal environmental conditions for infection may occur variably during the growing season. Moderately resistant cultivars had significantly lower AUDPCs than susceptible cultivars confirming that the most promising tool for Phomopsis management is genetic resistance.

Diseases of introduced *Eucalyptus* and native Myrtaceae in Uruguay: New cases of host jumping

C. A. PEREZ (1), M. J. Wingfield (2), B. Slippers (2), N. A. Altier (3), S. Simeto (3), R. A. Blanchette (1)
(1) Department of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA; (2) Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; (3) Instituto Nacional de Investigación Agropecuaria (INIA), Ruta 48, km 10, Canelones, Uruguay
Phytopathology 98:S124

Eucalyptus is one of the most extensively planted tree species in the world and Uruguay has tripled the area planted to it in the last 10 years. The expansion of *Eucalyptus* plantings, the presence of indigenous pathogens that could affect them and the threat of pathogens not yet in the country requires increased awareness and monitoring. Countrywide surveys were carried out in native Myrtaceae forests and *Eucalyptus* plantations to identify potential pathogens of these trees. Species were identified using a combination of morphological characteristics and DNA sequence comparisons to determine whether native pathogens were undergoing host shifts to infect *Eucalyptus*. Results suggest a strong relationship among diseases occurring in both introduced and native Myrtaceae. *Puccinia psidii*, *Quambalaria eucalypti*, certain species of Botryosphaeriaceae, as well as Mycosphaerellaceae were found infecting both introduced *Eucalyptus* and native Myrtaceous trees. Pathogenicity tests demonstrated cross pathogenicity to *Eucalyptus.* The presence of the same species on both native and introduced hosts indicates a strong biological exchange of pathogens. Further investigation is needed to better understand the movement of pathogens from native to introduced Myrtaceae and vice versa, and it should also consider the economic and ecological importance of this exchange. In addition, these results demonstrate the importance of investigating diseases of native Myrtaceae when characterizing populations of pathogens threatening *Eucalyptus* production. Continued investigations on the etiology of these diseases are needed in order to insure that sustainable *Eucalyptus* production in Uruguay can be realized.

First report of the *Eucalyptus* pathogen *Neofusicoccum eucalyptorum* on non-*Eucalyptus* hosts and preliminary estimation of its variability in Uruguay

C. A. PEREZ (1), M. J. Wingfield (2), B. Slippers (2), N. A. Altier (3), S. Simeto (3), R. A. Blanchette (1)
(1) Department of Plant Pathology, University of Minnesota, 495 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA; (2) Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; (3) Instituto Nacional de Investigación Agropecuaria (INIA), Ruta 48, km 10, Canelones, Uruguay
Phytopathology 98:S124

Neofusicoccum eucalyptorum (= '*Botryosphaeria*' *eucalyptorum*) exists as an endophyte in healthy *Eucalyptus* leaves, twigs and stems and causes cankers and die-back after the onset of stress. To date, *N. eucalyptorum* has been found only on *Eucalyptus* hosts. However, in this study we surveyed native and introduced Myrtaceae forests in Uruguay and found *N. eucalyptorum* on six different *Eucalyptus* species and on several species of native Myrtaceae. Monosporic cultures were identified based on conidial morphology and comparisons of DNA sequences for the ITS region of the rDNA operon. Isolates of *N. eucalyptorum* obtained from *Eucalyptus* and other Myrtaceae were then characterized to assess the extent of clonality and relationships among isolates from each host. Phenotypic characterization was performed by inoculating isolates on clonal seedlings of E. grandis. The genetic variability was assessed by using different molecular markers (ITS sequence, ISSR and SSR). Results reveal the occurrence of *N. eucalyptorum* associated with cankers and twig dieback of non-*Eucalyptus* hosts including *Blepharocalyx salicifolius*, *Myrceugenia glaucescens*, and M*yrrhinium atropurpureum* var. *octandrum*. In addition, phenotypic and genetic characterization indicates significant variability among isolates from the native Myrtaceae, but this variability was not clearly structured according to host species. These results expand the known host range for *N. eucalyptorum* and demonstrate the importance to include surveys and sample collection on native Myrtaceae to adequately characterize populations of *N. eucalyptorum* in Uruguay.

Ectopic expression of pepper potyvirus resistance gene pvr1-2 confers potato virus Y resistance in potato

K. PEREZ (1)
(1) Cornell University, Ithaca, NY, USA
Phytopathology 98:S124

Potato (Solanum tuberosum) is the fourth most important crop grown today. Widely cultivated potato varieties such as Russet Burbank, which covers more acreage in the U.S. than any other potato cultivar, are susceptible to diseases which can dramatically reduce production. Among them is potyvirus potato virus Y (PVY), an economically devastating virus found worldwide. Past transgenic PVY resistant potato varieties have used pathogen-derived sequences (PDR) which have been unaccepted in the marketplace. Rather than insert a viral gene, we have used a plant gene to confer resistance. In this study, we over-express two alleles of the naturally occurring pepper potyvirus resistance gene *pvr1*, which has been found to confer broad spectrum potyvirus resistance in transgenic tomato (Kang et al., 2007). Russet Burbank lines over-expressing the $pvr1^2$ allele from pepper were extremely resistant to all strains of PVY screened which included the four predominant PVY strains found in the US: PVY N, PVY NO, PVY O, and PVY NTN, the most destructive strain which causes tuber necrosis. Additionally, $pvr1^2$ plants grown from tubers of inoculated plants were completely virus free.

The virulence of *Banana bunchy top virus* in banana plants after injection with a bananacide

E. A. PEREZ (2), C. R. Hooks (2), K. Wang (2), M. G. Wright (2), R. P. Almeida (1), R. Manandhar (2)
(1) University of Berkeley; (2) University of Hawaii
Phytopathology 98:S124

Field experiments were conducted in commercial banana orchards on Oahu to determine how long after injection with a bananacide (i.e., herbicide registered for destroying banana plants) do *Banana bunchy top virus* (BBTV) infected banana plants remain virulent. For each trial, several symptomatic banana plants were randomly selected and injected with Roundup® herbicide at 2 ml per inch pseudostem diameter. Leaf or pseudostem samples and leaf or pseudostem disk samples were removed from the test plants just prior to herbicide injection and weekly until plant death (i.e. 6 weeks after injection). Additionally, virus-free banana aphids were allowed to feed on the leaf or pseudostem sample for 48 hours and then placed on known virus-free banana plants. Disk samples and aphids allowed to feed upon the samples were then tested using molecular technique (e.g., PCR) to determine if they were BBTV positive. We found that banana plants may remain virulent up to 6 weeks after herbicide injection. However, aphids that fed on the leaf samples tested positive and were able to transmit BBTV up to 5 weeks after injection, respectively. It was also discovered that Provado 1.6 F ® sprays prevented aphids from acquiring and transmitting BBTV to healthy plants for several days after application. Implications of these findings for managing virus spread and future research were discussed.

A multipathogen detection array for virus, viroid, fungal, and oomycete pathogens of solanaceous crops

K. L. PERRY (2), B. O. Agindotan (2), N. Zhang (1), C. Smart (1)
(1) Cornell University, Geneva, NY, USA; (2) Cornell University, Ithaca, NY, USA
Phytopathology 98:S124

A multipathogen detection array for the simultaneous detection of viruses, viroids, fungi, and oomycetes has been printed and tested. We previously reported the development of two complementary macroarrays for the detection of pathogens of solanaceous crops. The macroarray for the detection of viruses and viroids involved the randomly-primed amplification of pathogen genomic sequences. For the macroarray detection of fungi and oomycetes, a primer-specific amplification of sequences from the internal transcribed spacer of ribosomal RNA genes was employed. In both systems, the amplified products (targets) were chemically labeled and hybridized to spotted oligonucleotide probes on a nylon membrane. Membranes were prepared with 70-mer oligonucleotide probes for the detection of viruses/viroids and shorter 17- to 27-mers for the detection of fungi and oomycetes. To test the performance of the array, separate DNA and RNA extracts were prepared from a potato plant infected with four viruses (*Potato leaf roll virus*, *Potato virus S*, *Potato virus X*, and *Potato virus Y*) and *Phytophthora infestans*. Amplified targets for virus/viroid and fungi/oomycetes were prepared, labeled, combined in equal amounts and hybridized with a single macroarray membrane. All five pathogens were unequivocally detected. The macroarray for this work contained a total of ~250 probes for control plant sequences and 11 viruses, 1 viroid, and 43 fungi/oomycete pathogens. Additional probes for the detection of a total of ~125 viruses have been designed and included. Together, these probes represent most virus, viroid, fungal, and oomycete pathogens of solanaceous crops.

Phloem limitation of potato leafroll virus is an asset not a liability

K. PETER (1), P. Palukaitis (3), F. Gildow (2), S. Gray (4)
(1) Cornell University, Ithaca, NY 14853; (2) Pennsylvania State University; (3) Scottish Crop Research Institute; (4) USDA, ARS, Ithaca, NY 14853
Phytopathology 98:S124

Unlike most plant viruses, those that are grouped in the family *Luteoviridae* are restricted to phloem tissues and are considered to lack required movement proteins or plant anti-defense mechanisms that would allow them access to other host tissues. We report that potato leafroll virus (PLRV) actually selects

for a protein-mediated mechanism that limits virus to the phloem. PLRV and related viruses encode an extension of their major capsid protein translated inefficiently via a readthrough mechanism. This multifunctional readthrough domain when incorporated into the virion is required for aphid transmission. The readthrough protein also functions in *trans* as a movement protein by facilitating long distance movement and accumulation of virus in phloem tissue. Mutations that eliminate translation of the C-terminal half of the readthrough domain reduce the rate of virus movement in phloem and the number of infection foci. Unexpectedly, virus that is unable to translate either the entire readthrough domain or the C-terminal half of the domain was mechanically transmissible to *Solanum sarrachoides* (Hairy Nightshade), and in both inoculated and systemically infected tissue, the virus was able to escape its phloem limitation and infect mesophyll cells. Crystalline arrays of virions were observed associated with chloroplast membranes in mesophyll cells. Wild type virus was not mechanically transmissible and was observed only in phloem associated cells. However, the ability of the mutant viruses to move into mesophyll cells was short lived. Compensatory mutations were positively selected that restored translation of the entire readthrough domain and re-established the phloem-specific distribution of the virus. Phloem limitation of the luteoviridae is not a deficiency, but may have evolved to facilitate transmission and ensure continued dispersal to new hosts. A model for virus movement encompassing these observations will be presented.

***Tilletia indica*: Resiliency of allantoid sporidia and its relationship to wheat infection**

G. L. PETERSON (1), D. L. Glenn (1)
(1) USDA ARS NAA, Fort Detrick, MD, USA
Phytopathology 98:S125

Tilletia indica, causal agent of Karnal bunt of wheat, is a non-systemic bunt fungus that infects the wheat spikes at flowering via allantoid sporidia arising from basidiospores produced by germinating teliospores on the soil surface. Historically, secondary sporidia have been reported as fragile, requiring high levels of sustained moisture to survive and infect. A recent invitro study however, demonstrated that allantoid sporidia of *T. indica*, as well as a number of other *Tilletia* species remained viable after an extensive period of dehydration and low humidity. The aim of this study was to examine the significance of this discovery on the infection process. In this study, repeated three times, groups of wheat spikes (cv. Cavalier) were spray inoculated with a sporidial suspension at two concentrations (2.0×10^3 or 1.0×10^4 sporidia per ml, 1.0 ml per spike). For each concentration, a set of 20 inoculated spikes were placed overnight in a dew chamber at 18C immediately after inoculation or at 24, 48, 120, 336 h after inoculation. When not in dew, plants were kept in the greenhouse (mean 23C and 27% RH). Twenty inoculated spikes were never placed in dew. At maturity, the number of infected seeds per spike was determined for each treatment. Mean (std dev) percent seed infection per spike was 43.42 (12.38), 26.26 (22.34), 11.29 (12.00) 3.21 (5.57), and 0, at the higher concentration and 43.87 (24.64), 33.82 (4.43), 18.38 (15.70) 8.41 (0.02), and 0.0, at the lower concentration for dry periods of 0, 24, 48, 120 and 336 h between inoculation and exposure to moisture conditions favorable for infection, respectively. Inoculated plants never exposed to overnight dew did not become infected.

Defense genes and pathways in Fusarium crown rot susceptible and partially-resistant Australian wheat seedlings responding to *Fusarium culmorum* infection

J. E. PETRISKO (1), J. M. Windes (1)
(1) University of Idaho
Phytopathology 98:S125

Fusarium crown rot causes considerable losses to wheat production in parts of the United States and Australia. Australian wheat germplasm has been the best source of resistance to Fusarium crown rot caused by *F. pseudograminearum*. In order to understand the mechanism of resistance to Fusarium crown rot caused by *F. culmorum*, a transcriptional study using the Affymetrix wheat chip was done to compare the partially-resistant Australian line '2-49' to the susceptible Australian wheat variety 'Puseas' at ten days post-inoculation. Genes determined to be significantly different in expression levels were identified in four categories: 2-49 inoculated versus 2-49 non-inoculated, Puseas inoculated versus Puseas non-inoculated, 2-49 inoculated versus Puseas inoculated and 2-49 non-inoculated versus Puseas non-inoculated. Five candidate genes, oxalate oxidase, chitinase 1, glycosyltransferase, WIR1, and a stress-related protein were selected for further expression characterization at one, five, and ten days post-inoculation using quantitative real-time RT-PCR. The quantitative real-time RT-PCR results confirmed the microarray results and showed that differential expression in these genes was due to differences in genetic background, inoculation response, or the interaction of both factors. These genes are being investigated for their roles in the resistance response of 2-49 using a viral-induced gene silencing (VIGS) study and pathways analysis. An understanding of how 2-49 prevents *F. culmorum* spread throughout the seedling will greatly improve our understanding of resistance to fungal invasion in wheat.

Late winter climatic conditions influence ascospore production and release in *Venturia inaequalis*

V. PHILION (3), A. Stensvand (1), H. Eikemo (1), D. M. Gadoury (2)
(1) Bioforsk Norwegian Inst of Agric & Env Res, Aas 1432, Norway; (2) Cornell University, New York State Agricultural Experiment Station, Geneva, NY, USA; (3) IRDA, Mont-Saint-Bruno, Québec
Phytopathology 98:S125

Most fungicide applications targeting apple scab aim to control primary infections caused by ascospores and spraying is thereby linked to ascospore availability. We investigated the effect of pre bud break climatic conditions on seasonal patterns of ascospore release. Apple leaves bearing pseudothecia of *Venturia inaequalis* were overwintered at orchard sites in 8 countries for up to 3 years. Leaf samples were collected 2 to 5 weeks before bud break and again at bud break, air dried, and sent via airmail to Norway. The samples were stored at –18°C upon arrival until tested. Disks cut from each replicate leaf sample were incubated moist at 20°C to allow ascospore maturation but prevent discharge. Matured ascospores were induced to discharge twice a week and enumerated until the supply was exhausted. The proportion of ascospores ejected was fitted against degree day accumulation using logistic regression. The regression intercept (onset maturation), slope (maturation rate), as well as the absolute number of spores counted differed significantly ($P < 0.001$, $P = 0.05$, $P < 0.001$ respectively) among sites and sampling dates. There was a significant interaction between site and sampling date, indicating that climatic conditions prior to bud break differentially impacted the subsequent ascospore availability. Observed differences could perhaps be used to further refine previously described models of ascospore maturity.

Soybean rust incidence and the response of soybeans to fungicides in Virginia 2007

P. Phipps (1), E. STROMBERG (2), S. Rideout (3), D. Holshouser (1)
(1) Virginia Polytechnic Institute & State University, Suffolk, VA; (2) Virginia Polytechnic Institute & State University, Blacksburg, VA; (3) Virginia Polytechnic Institute & State University, Painter, VA
Phytopathology 98:S125

Soybean leaflets were collected and incubated for 3–5 days from 10 sentinel plots and 84 commercial fields for detection of soybean rust in 2007. Sentinel plots were located at Tidewater Agricultural Research and Extension Center (AREC) (Suffolk), Southampton County, Eastern Shore AREC (Painter), Northampton County, Shenandoah County, Southern Piedmont AREC (Blackstone), Charles City County, Northern Piedmont AREC (Orange), Eastern Virginia AREC (Richmond County), and Virginia Tech – Kentland Farm (Blacksburg). The first outbreak of soybean rust, caused by *Phakopsora pachyrhizi*, was found in leaflets from Isle of Wight County on 19 October; thereafter, disease was confirmed in 9 counties and cities (Chesapeake, Gloucester, Isle of Wight, Matthews, Middlesex, Suffolk, Surry, Sussex, and Virginia Beach). No loss of yield to soybean rust was expected since the disease appeared when soybeans were beyond growth stage R6 (full seed). Above normal temperatures and below normal rainfall in July, August, and September suppressed yield and resulted in unfavorable conditions for soybean rust; except for 16 of 22 days from July 12 to Aug 2 and 16 of 22 days from 20 September to 8 October. Dry weather stress during the season limited development of common diseases in soybeans throughout most of the season (i.e. cercospora blight, purple seed stain, brown spot, frogeye leaf spot, anthracnose, pod and stem blight, etc.). Fungicide treatments showed little or no effect on yield in nine replicated field trials in 2007.

PI424487B has at least 2 *Rps* genes that confer resistance to *Phytophthora sojae*

W. PIPATPONGPINYO (2), S. Berry (2), S. K. St. Martin (1), A. E. Dorrance (2)
(1) The Ohio State University, Columbus, OH; (2) The Ohio State University, OARDC, Wooster, OH
Phytopathology 98:S125

New *Rps* genes that are effective against *Phytophthora sojae* are needed to manage this pathogen effectively in soybean. Soybean plant introductions (PIs) from South Korea were identified as putative new sources for novel *Rps* genes including PI424487B. The objective of our study was to characterize the *Rps* genes in this PI. Individual lines from two generations ($F_{2:3}$ and $F_{2:4}$) of Williams × 424487B population were inoculated with *P. sojae* race 17 (vir 1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6, 7) and race 25 (1a, 1b, 1c, 1k, 7) and at least two genes conferred resistance to each isolate. From the $F_{2:4}$ population, 187 lines were then screened with SSRs from 12 different molecular linkage groups (MLG). Based on single marker association, resistance to race 17 was associated with SSRs on MLG F ($P < 0.001$), N ($P < 0.0001$) and B1 ($P <$

0.05). There was also a significant association with resistance to Race 25 with SSRs from MLG F($P < 0.01$). Based on phenotypic and genotypic analysis, PI424487B has at least 2 *Rps* genes at MLG N and F in regions which contain numerous other resistance gene loci.

A new disease of *Syzygium paniculatum* (Myrtaceae)
R. C. PLOETZ (1), J. M. Perez-Martinez (1), A. J. Palmateer (1)
(1) University of Florida, Homestead, FL, USA
Phytopathology 98:S126

Syzygium paniculatum, a native of Australia, is an important plant in the South Florida ornamental and topiary industry. Also known as eugenia and brush cherry, the plant was relatively free of diseases before Hurricane Wilma (2005). Since then, a serious dieback has become prevalent in local nurseries; symptoms include wilting and death of terminal and/or lateral branches, branch mortality without defoliation, and extensive vascular discoloration. Diverse fungi have been isolated from diseased and healthy plants, but *Botryosphaeria*-like fungi predominate, including: putative species of *Fusicoccum* and *Neoscytalidium*; a homothallic *Botryosphaeria* sp. that does not form an anamorph; and isolates that produce the steely grey, cottony mycelium of the *Fusicoccum* sp. on PDA, sclerotial-like structures on toothpicks and pine needles, but are otherwise sterile. After artificial inoculation, only the later fungi caused disease. In incubator studies, mortality developed only at 30C, moderate damage occurred at 25C and no disease developed at 20 or 15C. Ongoing work will: i) identify the various taxa with DNA sequences, ii) determine sources/reservoirs of the pathogen, iii) investigate host infection and colonization, iv) examine the susceptibility of different cultivars of *S. paniculatum*, and v) assess factors that may contribute to the development of this disease, including light intensity, fertility, and irrigation practices.

Recovery and functional analysis of six contiguous genes that may affect parasitic fitness in the Dutch elm disease fungus *Ophiostoma novo-ulmi*
K. V. PLOURDE (1), V. Jacobi (1), L. Bernier (1)
(1) Laval University CEF, Quebec, Canada
Phytopathology 98:S126

The ascomycete fungus *Ophiostoma novo-ulmi* is the main causal agent of Dutch elm Disease. We used insertional mutagenesis to tag genes that may contribute to parasitic fitness in this fungus. We recovered several transformants with a significantly reduced pathogenicity phenotype compared to the aggressive wild-type reference strain H327. Analysis of a genomic library for one of these transformants allowed us to identify six genes next to the mutation site. To correlate differences in the expression of these genes between the transformant and H327 with differences in their ability to colonize elms, both were grown on elm sapwood agar (ESA), a substrate mimicking the host environment. Real time PCR indicated that uridine nucleosidase (urh1) and ubiquinol-cytochrome C reductase were significantly down regulated and a hypothetical protein up regulated in the transformant. Our results suggest theses genes could be implicated in the parasitic fitness of *O. novo-ulmi* towards elms.

Incidence, severity and management of Cytospora canker in stone fruits
R. R. POKHAREL (1), H. Larsen (1)
(1) Western Colorado Research Center
Phytopathology 98:S126

Peach, cherry, plum, and apricot orchards in western Colorado were surveyed for incidence and severity of Cytospora canker using a disease severity rating scale of 1 to 9 (1 = healthy and 9 = dead). All orchards surveyed had Cytospora infection (15 to 100% of trees within each orchard) with severity ratings ranging between 1.6 to 5.6 across crops, varieties, tree age, and management approach (conventional vs organic). Berenda Sun, June Pride, and Elegant Lady peaches, Bing sweet cherry, and Stanley European plum had the highest incidence and severity. Poultices of seed meal + ground leaf material of 1) brown mustard (*Brassica juncea*), 2) yellow mustard (*B. hirta*), or of 3) canola (*B. napus*) seed meal mixed with root and leaf material of horseradish (*Armoricia rusticana*) were applied to naturally infected cankers during fall 2007. Gum production (evidence of canker activity) lessened or dried up during the 2007 growing season on cankers treated with either the brown mustard or the canola/horseradish mix. Bark samples were collected from treated and non-treated cankers and plated out onto PDA media. The causal fungus was recovered and grew well from the non-treated cankers but not from treated cankers. Further studies of *Brassica* materials and other chemicals for Cytospora management for organic and conventional growers are in progress.

Reassessment of the taxonomic classification of the cranberry pathogen *Physalospora vaccinii*
J. J. POLASHOCK (3), P. V. Oudemans (1), J. Crouch (2)
(1) Rutgers University, Chatsworth, NJ, USA; (2) Rutgers University, New Brunswick, NJ, USA; (3) USDA-ARS, Chatsworth, NJ, USA
Phytopathology 98:S126

Blotch rot of cranberry is caused by the ascomycete *Physalospora vaccinii*. This pathogen is widespread and has been reported from all cranberry growing regions in North America. The frequency of isolation from rotted fruit can be as high as 7% in New Jersey. This species exhibits two different morphological types: the most common is characterized by gray colonies and profuse sporulation, while the minority morphotype is distinguished by white colonies with minimal spore production. Phylogenetic analysis of ITS sequences shows the two morphological types are separated into distinct clades, each comprised of diverse genotypes, consistent with a long-established, sexually reproducing species. Despite this well-supported distinction, the relationship between the two clades is best characterized by a reticulating network topology, with evidence of gene flow between the two populations. Our phylogenetic analyses of rDNA ITS and LSU sequence data challenge the morphologically-based classification of *P. vaccinii* within the Sordariomycete subclass Xylariomycetidae, as extant isolates of *P. vaccinii* are more closely allied with members of the subclass Sodariomycetidae. These data are sufficient to question whether the blotch rot pathogens of cranberry are actually members of the genus *Physalospora*. Here we present multilocus phylogenetic analyses in combination with morphological analyses of authentic *P. vaccinii* specimens to reexamine the true identity of the blotch rot pathogen of cranberry.

Development of biologically-based management strategies for postharvest disease control on apples
A. M. POLEATEWICH (1), P. A. Backman (1), J. Travis (1)
(1) Penn State University, University Park, PA
Phytopathology 98:S126

Synthetic fungicides remain the primary means used to control postharvest diseases of fruits and vegetables. Considerable attention has focused on the potential use of biological controls for postharvest disease management. Many organisms, while successful in research trials, are difficult to formulate and many never reach the marketplace. Sporulating gram-positive bacteria such as Bacillus spp. and Streptomyces spp. produce spores resistant to heat and desiccation that can easily be formulated as a dry product. The objective of this research is to investigate the ability of beneficial endospore-producing bacteria, collected from apple and vegetable crops, used alone or in combination with amendments, to suppress postharvest diseases on susceptible apple varieties. Bacteria were collected from abandoned, low input, organic, and conventionally managed orchards in PA and were screened for their ability to produce chitinase enzymes, and colonize apple leaves and fruit. Fruits of cultivars 'Rome Beauty' and 'Fuji' were spray inoculated with 9 bacterial isolates with or without 2% AgSil for 18 replicates. Apples were stored in growth chambers at 19°C and 70% RH. One week later, fruit were wounded with a 6p nail to a depth of 3 mm. Wounds were inoculated with 20 µl of the bacterial isolate and 20 µl of a 104 conidial suspension of Colletotrichum actuatum one hour later. Lesion diameter was measured one week after pathogen challenge. Several isolates reduced bitter rot lesion size on 'Rome Beauty' from 40–89% compared to untreated controls. Development of postharvest biological controls will reduce dependence on fungicides and limit human exposure to harmful residues on fruit.

Effects of soybean cyst nematode on growth of pinto bean
S. POROMARTO (1), B. D. Nelson (1)
(1) Dept. Plant Pathology, NDSU, Fargo, ND
Phytopathology 98:S126

Phaseolus vulgaris is a host of the soybean cyst nematode (SCN; *Heterodera glycines*), but the effects of SCN on growth of the plant are unknown. The effect of SCN (HG type 0) on growth of pinto bean 'GTS-900' was evaluated in the field in 2007. Soil from a naturally infested field was pasteurized and re-infested with 0, 5,000 or 10,000 SCN eggs/100cc soil. Soil was placed in 10 L plastic pots that were buried in the field with the bottoms removed. Individual plants were grown in pots with 6 replications per treatment and plants were harvested when mature. Plant height, pod number, pod weight, seed number, seed weight, and total dry weight of the above-ground-plant were significantly ($P < 0.05$) less in the two SCN treatments compared to the control, but there were no significant differences between the two SCN treatments. Total seed weight from the infected plants was only 44% of that in the control. The number of cysts and eggs in the soil at harvest was significantly ($P < 0.05$) higher in the 10,000 compared to the 5,000 eggs/100 cc soil infestation, and both were significantly higher compared to the control. This is the first data from controlled experiments demonstrating reduction in growth of a dry bean cultivar by SCN. SCN is a potential threat to dry bean production in the northern bean growing areas.

Prolonged infection periods to *Sclerotinia sclerotiorum* identified in wild pea germplasm to be breed into pea cultivars to promote disease avoidance

L. D. PORTER (1), V. A. Coffman (1)
(1) USDA-ARS, Prosser, WA, USA
Phytopathology 98:S127

White mold, caused by *Sclerotinia sclerotiorum*, is a foliar pathogen that can cause serious disease in irrigated and dryland peas. Management of white mold in peas with fungicides is not economical and resistant cultivars do not exist. Therefore, 504 wild pea accessions were screened for resistance to *S. Sclerotiorum*. Although all 504 lines were susceptible to infection, ten of the most resistant lines to lesion expansion were selected for further studies. The selected lines were compared to the susceptible pea cultivar Bolero for length of infection period and lesion expansion when plants were inoculated and maintained at 24 different combinations of temperature (15.6, 18.3, 21.1, 23.9, 29.4°C) and incubation period (12, 24, 48, 72 hours) in a growth chamber at 90–100% RH. All pea lines screened at a 12-hour incubation period prevented lesion expansion beyond the inoculation point at all temperatures tested except 1204-3, 166084 and Bolero. Pea lines screened at a 24-hour incubation period were all highly susceptible to infection and lesion expansion at all six temperatures except for lines, 103709, 164972, and 169603 were resistant at 29.4°C, and 169603 was resistant at 23.9°C. No pea line was resistant to infection at any of the temperatures when incubated for greater than 24 hours. Future efforts will concentrate on identifying quantitative trait loci associated with prolonged infection periods that can be used in marker-assisted selection for this trait. Identified lines will be crossed with semi-leafless upright pea cultivars to create new cultivars with prolonged infection periods that will maximize disease avoidance and have upright semi-leafless canopies that will limit environmental conditions favoring white mold.

Alternative control of *Rhizoctonia solani* on potato crop by using Rhizomarr (potassium permanganate at 18% ce) in Tapalpa, Jalisco, México

P. POSOS-PONCE (1), J. L. Martinez Ramirez (1), G. Enciso-Cabral (1), C. Duran-Martinez (1), L. A. Rendon-Salcido (1)
(1) Universidad de Guadalajara
Phytopathology 98:S127

There were carried out two field trials to evaluate the product Rhizomarr (potassium permanganate) on potatoe crop Alfa variety for controlling "Costra Negra" (*Rhizoctonia solani*). Three Rhizomarr dosages were evaluated (1.0, 2.0 and 3.0 L/ha.) and comparing with Rizolex (Tolclofos metil 6 kg/ha.). Treatments were applied at sowing time. The parameters evaluated were damage and biological effectiveness at 50 DAA. At the end of the trial were evaluated yield. Results showed that best dosage for biological effectiveness it was Rizolex (Tolclofos metil) obtaining 75% of conttol. The best Rhizomarr dosage it was 6.0 L/ha. obtaining 61% of control. Using treatment 3 of Rhizomarr (3.0 L/ha.) was obtained the highest number or tuber by lineal meter. It is recomendable to apply Rhizomarr at 2 and 4 L/ha. dosage which it cuould be increase until 5 L/ha at the sowing time on potatoe crop. On the control check it was present around 20% of *Rizoctonia solani*. There was not observed phytotoxicity on the potato crop.

Bacillus amyloliquefaciens KPS46 produces indole-3-acetic acid in culture medium and its effect on growth promotion via increased proteome expression and indole-3-acetic acid content in soybean plant

S. PRATHUANGWONG (1), N. Buensanteai (1)
(1) Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand
Phytopathology 98:S127

Bacillus amyloliquefaciens KPS46 was reported to successively control several plant diseases and enhance plant growth in greenhouse and field conditions. The microbial ability has been reported to result directly from its production of phytohormone, including indole-3-acetic acid (IAA). IAA-PGPR producers colonizing the seed surfaces is proposed to act in conjunction with endogenous IAA in plant's IAA complex to control many important physiological and biochemical processes including cell enlargement, division, and tissue differentiation. This study focused on determining auxin produced by KPS46 that may associate with its plant growth promotion. The KPS46's IAA obtained from NGB detected by HPLC was highly secreted at stationary phase after 48 h incubation with 31.0 ug of IAA ml-1. KPS46 culture produced higher amounts of IAA when added tryptophan with moderated stress of 0, 250, 500, 2500 and 5000 mM was incorporated in the mineral culture medium. Moreover, the efficacy of KPS46 in promoting soybean growth was confirmed which it could increase root length, shoot length, dry weights, and number lateral root by more than 41, 22, 33, 23% respectively compared to the negative control. Inoculation of soybean seeds with KPS46 enhanced two fold of the plant's IAA content of non-treated control. We also focused on proteomic approach, soybean leaf proteins were separated at 14 days after KPS46 inoculation. The total proteins were analyzed by 2D-PAGE and identified amino acid sequence by MADI-TOF-MS. The result revealed, 20 selected proteins that were up-regulated by KPS46 may fall into several functional categories. These proteins may relate to growth and development of soybean plant after primed with the strain KPS46.

Occurrence, survival, and population levels of *Trichoderma virens* in soils of animal waste application sites in Mississippi

R. G. PRATT (1)
(1) USDA, ARS, Mississippi State, MS
Phytopathology 98:S127

Population levels of *Trichoderma virens*, a plant disease biocontrol species, were assayed in soils from three farms in Mississippi where swine waste was applied to pastures, and from two farms where poultry litter was applied as a fertilizer to cotton. Assays of soil from each site with and without applied waste or litter were performed by dilution plating on Sabouraud's medium plus chloramphenicol. Soils were assayed shortly after collection and after storage for up to 5 years in sealed bags. *T. virens* was detected from fresh or stored soil from all sites at mean population levels of $0.07\text{-}1 \times 10^4$ cfu/g soil in positive samples. These represented 8–100% of total *Trichoderma* populations. Population levels of *T. virens* differed between sites and waste application treatments in some soil samples. *T. virens* was detected frequently in soils stored for up to 2 years, and it survived for 5 years in soil from one site. Results indicate that *T. virens* is widely distributed in Mississippi soils, and that population levels may differ between sites and in response to animal waste application practices.

Distribution and control of *Pseudocercospora angolensis* on citrus in Zimbabwe and Mozambique

M. C. PRETORIUS (1), G. Holtz (2)
(1) Citrus Research International, Nelspruit 1200, Mpumalanga, South Africa; (2) Department of Plant Pathology, University of Stellenbosch, Matieland 7602, South Africa
Phytopathology 98:S127

Leaf samples with citrus canker-like lesions collected in the early 1990's in Zimbabwe were found to be infected by the fungus *Pseudocercospora angolensis*, causative organism of Fruit and Leaf Spot Disease (FLSD) on citrus. Three surveys were undertaken in Zimbabwe. The survey in Mozambique was undertaken from the southern border to the Beira corridor. It was found that *P. angolensis* in Zimbabwe was limited to an area above the 19° south latitude, predominantly the moist areas and not the low-lying drier parts of the country. In Mozambique, no *P. angolensis* symptoms were noted. The disease cycle coincided with high rainfall during the warmer summer months. The control strategy consisted of eradication of infected trees and neglected orchards for the reduction of inoculum, and implementation of an effective spray programme. The evaluation consisted of applying several fungicides at different rates and dates. The trifloxystrobin + mancozeb + mineral spray oil (20 g + 200 g + 500 millilitres/100 litres water) treatment sprayed during November, January and March reduced *P. angolensis* most effectively and resulted in the highest percentage flushes (72.8%) free from symptoms. Applications during November and January of difenoconazole (40 millilitres/100 litres water) resulted only in 55.4% leaf flushes free from symptoms. Mancozeb (200 g/100 litres water), applied three times during the season, indicated that a contact fungicide applied during the rainy season performed poorly. Once neglected orchards are removed, the spread and intensity of FLSD in the remaining trees can be reduced if a well-managed and a timely spray programme is implemented, thus resulting in producers producing lesion-free fruit without the associated phytosanitary risk.

***Pseudocercospora angolensis*, the cause of fruit and leaf spot disease of citrus in Zimbabwe**

M. C. PRETORIUS (2), P. W. Crouse (1), G. Holtz (3)
(1) Centraalbureau voor Schimmelcultures, 3584 CT Utrecht, The Netherlands; (2) Citrus Research International, Nelspruit 1200, Mpumalanga, South Africa; (3) Department of Plant Pathology, University of Stellenbosch, Matieland 7602, South Africa
Phytopathology 98:S127

Fruit and leaf spot disease (FLSD) of citrus, previously known as *Phaeoramularia angolensis*, is predominantly found in sub-Saharan Africa, the Comoro Islands, and has also been reported from Yemen on the Arabian Peninsula. The disease has been observed on all citrus cultivars. It is a disease of major phytosanitary and economic importance for citrus producing countries in Africa. The damage is cosmetic with the development of fruit spots which renders the fruit unmarketable. Yield losses of 50–100% are common in Kenya. The disease is presently restricted to two areas north of Harare in Zimbabwe. On leaves the fungus produces a circular spot approximately 10 mm in diameter. Spots generally occur singly and are surrounded by a prominent yellow halo. On fruit, the infected areas are circular to irregular, discrete or coalescent, and surrounded by yellow halos.

Young fruit symptoms often commence with nipple-like swellings without a yellow halo. Spots on mature fruit are normally flat and often surrounded by a dark brown to black sunken margin of anthracnose. Prolonged wet weather with moderate temperatures of 22–26°C, favour the disease. Lesions on older leaves sporulate 3–5 weeks after commencement of the rainy season and symptoms on young leaves appear 2–3 weeks later. This suggests that conidia produced the previous season infect new flush and the disease cycle continues. Long distance dispersal of the fungus is by windborne conidia. The causal organism of FLSD of citrus was initially identified as *Cercospora angolensis*. The species was later transferred to *Phaeoramularia*. The fungus forms dense tufts (synnemata) of light chestnut-coloured, multiseptate conidiophores, which arise from a stroma and emerge through stomata on the lower leaf surfaces, bearing conidia singly or in chains of two to four. Recent molecular studies showed that the genera with such scars as in *Paracercospora* and *Pseudophaeoramularia* belong in *Pseudocercospora* and a new combination for Cercospora angolensis is herewith proposed in *Pseudocercospora*.

Microbial enrichment of compost with *Trichoderma* sp. to enhance suppressiveness against *Rhizoctonia solani*
M. PUGLIESE (1), A. Garibaldi (1), M. Gullino (1)
(1) Agroinnova - University of Torino, Via L. da Vinci 44, 10095 - Grugliasco (TO), Italy
Phytopathology 98:S128

Compost can suppress soil-borne plant diseases, avoiding the use of peat, chemical pesticides and fertilizers. An increase of some diseases due to compost usage has also been demonstrated, since compost is a product that varies considerably in chemical, physical and biotic composition, and, consequently, also in ability to suppress soilborne diseases. In particular, the capacity of composts to suppress *Rhizoctonia solani* remains limited. The aim of the research was to assess the effect of the enrichment with biological control agents on the level and reproducibility of suppressive properties of compost. A compost produced in Piedmont Region, in the North-West of Italy, made from green wastes, organic domestic wastes and urban sludges was inoculated with three commercial formulates of *Trichoderma* at 1, 2 and 4 g/l dosages and the effect on the pathosystem *R. solani*/ bean was assessed in greenhouse trials. Pure compost showed to be not suppressive against *R. solani.* Only *Trichoderma harzianum* T-22 Rifai (Rootshield granules, Bio Works Inc., USA) at 4 g/l dosages showed an increase in the disease suppressiveness of compost up to 30% compared to the inoculated control. An increase in biomass of bean up to 29% compared to inoculated control was also showed. The other microorganisms tested did not increased the disease suppressiveness of compost.

Antibiosis and acidification by *Pantoea agglomerans* strain E325 may contribute to suppression of *Erwinia amylovora*
P. PUSEY (2), V. O. Stockwell (1), D. R. Rudell (2)
(1) Oregon State University, Corvallis, OR, USA; (2) USDA-ARS, Wenatchee, WA, USA
Phytopathology 98:S128

Pantoea agglomerans strain E325, a commercially-available antagonist for fire blight of apple and pear, was originally selected through screening based on suppression of *Erwinia amylovora* on flower stigmas, but specific mechanisms of antagonism were unknown. Bacterial modification of pH was evaluated as a possible mechanism by analyzing stigma exudates extracted from 'Gala' apple stigmas. The pH values for field samples were only slightly lower than controls, but indicated a range (pH 5-6) conducive for antibiotic activity according to subsequent assays. Under low-phosphate and low-pH conditions, an antibacterial product of E325 with high specificity to *E. amylovora* was effective at low concentrations. A minimum of 20 to 40 ng of a ninhydrin-reactive compound purified using RP-HPLC caused visible inhibition in assays. Activity was heat stable and unaffected by amino acids, iron, or enzymes known to affect antibiotics of *P. agglomerans*. Antibiosis was diminished, however, under basic conditions, and with increasing phosphate concentrations at pH 6 and 7. Inhibition was not observed in media containing phosphate concentrations commonly used in antibiosis assays. We propose that E325 suppresses the fire blight pathogen not only by competing for nutrients on the stigma, but by producing an antibiotic specific to *E. amylovora*. Further work is necessary to substantiate that the compound is produced and active on flower stigmas.

Salinity-induced predisposition to *Phytophthora capsici* in abscisic acid-deficient tomato seedlings
M. F. PYE (1), M. Dileo (1), R. Bostock (1)
(1) UC Davis, Davis, CA, USA
Phytopathology 98:S128

Episodes of water deficit and soil salinity are common abiotic root stresses that can increase the incidence and severity of diseases caused by *Phytophthora* species. Abscisic acid (ABA) increases rapidly in roots and shoots under conditions of water deficit and remains elevated for hours after removal of the plants from the stress. Modification of ABA levels in tomato seedling roots by exogenous application of ABA or by osmotic stress increases their susceptibility to *Phytophthora capsici*. To further evaluate the role of ABA in root stress predisposition, hydroponically-grown ABA-deficient tomato lines, *sitiens*, *flacca* and *notabilis*, were exposed for 6 hr to osmotic stress with NaCl and their susceptibility to *P. capsici* compared to similarly stressed wild-type tomato seedlings. Roots were inoculated following removal of the plants from NaCl and transfer back to normal hydroponic medium. Roots and shoots of the ABA-deficient mutants were shown to accumulate significantly lower levels of ABA than wild-type plants following a stress event, as determined by GC-MS. Analyses of ABA levels and disease severity in roots of ABA-mutant and wild-type tomatoes during predisposition onset and recovery will be presented.

Spatial distribution of *Sclerotinia sclerotiorum* ascospores and its relation to sclerotinia stem rot of canola
I. S. QANDAH (1), L. E. Del Rio (1)
(1) North Dakota State University, Fargo, ND, USA
Phytopathology 98:S128

Seasonal and daily patterns of ascospore dispersal of *Sclerotinia sclerotiorum*, causal agent of sclerotinia stem rot (SSR) of canola, were studied in commercial canola fields in North Dakota from mid June, until the first week of August in 2005, 2006, and 2007. Ascospores were collected on Petri plates containing a semi-selective medium at various distances from an area source of inoculum. These plates, placed on soil surface under plant canopy, were exposed for three hours between 10:00 A.M and 1:00 P.M. Peaks of high ascospore concentrations were detected when the air temperature and relative humidity under plant canopy were 13–20°C and >85%, respectively. An exponential regression models described the relationship between the mean number of colonies/week trapped and SSR incidence ($r^2 > 0.94$ and $Pr > 0.004$) in both location in 2005, and 2007. In 2006 we were not able to trap any ascospores during the same period under the plant canopy, and SSR did not develop in the field. Inoculum concentration decreased with distance from the source ($r^2 > 0.89$ and $Pr > 0.03$). 50% reduction in SSR incidence was detected at 20 meter going away from source of inoculum ($r^2 > 0.94$ and $P > 0.002$). Also 50% reduction in the mean number of colonies trapped under plant canopy was detected at 20 meter away going form the inoculum source ($r^2 > 0.89$ and $Pr > 0.03$).

Study on the resistance risk and resistance inheritance of *Phytophthora capsici* to flumorph
M. Qinxiao (1), C. Xiaolan (1), B. Yang (1), L. Xiaohong (1), H. Jianjun (2), L. XILI (1)
(1) Department of Plant Pathology, China Agricultural University, Beijing, R.P. China; (2) Department of Plant Pathology, Michigan State University, 62 Plant Biology Bldg, East Lansing, MI 48824
Phytopathology 98:S128

Flumorph, a carboxylic acid amide (CAA) fungicide, is highly effective to control some oomycetes pathogens. Study of *P. capsici* on potential resistance risk to flumorph was conducted. Thirteen flumorph-resistant mutants of *Phytophthora capsici* were obtained by treating mycelia and zoospores from A2 parental strain with ultraviolet rays. One mutant exhibited a high level of resistance with factors of 216.67 folds. This was the first to show the existence of potential development of resistance to CAA fungicides in *P. capsici*. The resistance of all flumorph-resistant mutants was stable after subculture for 10 times. Comparing to parental strains, the resistant mutants had no significant difference in mycelial growth and sporulation *in vitro*, and pathogenicity on detached leaves. Most flumorph-resistant mutants showed a high fitness of living. There was cross-resistance among flumorph, dimethomorph and iprovalicarb, but not with azoxystrobin, cyazofamid, cymoxanil, or metalaxy. These studies suggested that *P. capsici* had potential laboratory resistance risk to flumorph. Combined the above results and the characteristic *P. capsici* is soil borne, the resistance risk can be classified as moderate. Appropriate precautions against resistance development should be taken. In the mean time the inheritance of resistance to flumorph was investigated, and the preliminary result revealed that there were natural resistance strains in the self-cross progeny from some wild strains of *P. capsici*. It suggested that the resistance of *P. capsici* to CAA fungicides was likely controlled by recessive nuclear gene.

Using the antagonist control a soil-borne wilt of cabbage
J. QIU (1), J. Liu (1), W. Liu (1), C. Lu (1), T. Liu (1)
(1) Institute of Plant & Environment Protection, Beijing Academy of Agri. & Forestry Sci., Beijing China
Phytopathology 98:S128

Cabbage wilt disease developed with high speed in Beijing recently and cultures of some microbes have been found to inhibit plant pathogens broadly

and efficiently. Some of microbes displayed a broad-spectrum antagonistic activity to soil-borne pathogens tested in the study. *Clonostachya rosea* ACM941, *Trichoderma viride* GZ-1, *Bacillus subtilis* B03 and *Panibacillus polymyxa* T99 were used to test their ability of controlling of *Fusarium oxysporum* f. sp. *conglutinans*, in cabbage field. The fermented broth of them were played an ability of growth competition, re-parasitic and inhibiting mycelium growth of *F. oxysporum* f. sp. *conglutinans*. The potted test with fermented broth presented an excellent bio-control effect on *F. oxysporum* f. sp. *conglutihans* and the highest control efficiency reached 96.25%. Also plant roots treated with 4 broth of above for Fusarium wilt of cabbage showed both low disease index and low infection rate. Treating with broth by the method of drenching or dipping, after transplanting, and a further three times during the growing season. In contrast, the control rate at the spring season were 78.5–94% from *Clonostachya rosea* ACM941, 69.3–83.9% from *Trichoderma viride* GZ-1, 86% from *Bacillus subtilis* B03, 70.1–76.1% from *Panibacillus polymyxa* T99 respectively.

Fraser fir, a new host of *Phytophthora capsici*

L. M. QUESADA OCAMPO (1), D. W. Fulbright (1), M. K. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, USA
Phytopathology 98:S129

Phytophthora cinnamomi, *P. drechsleri*, *P. citricola* and *P. cactorum* limit Fraser fir production whereas *P. capsici* affects primarily vegetable crops. Our objectives were to determine whether *P. capsici* can infect and cause disease on Fraser fir. Seedlings were stem-inoculated (no wound or 1- or 3-mm-diameter wound) with *P. capsici* isolate OP97 and incubated in growth chambers at 20 or 25°C. Four diverse isolates were used to stem-inoculate seedlings (no wound or 1-mm-diameter wound) incubated at 25°C. Seedlings were soil-inoculated (2 or 4 g of infested millet seed and 2 or 5 × 10^3 zoospores/ml of a zoospore solution) with OP97 and incubated at 25°C. Four isolates were used to soil-inoculate seedlings (2 or 4 g of infested millet seed) incubated at 25°C. Four hundred seedlings were planted in vegetable fields with a known history of *P. capsici*. Experiments were conducted twice and controls were included. All *P. capsici* isolates infected inoculated seedlings despite the inoculation method or incubation temperature used. Seedlings planted in the field presented symptoms and died (72%). The pathogen was reisolated from symptomatic seedlings and the phenotype (mating type and mefenoxam resistance) was confirmed for inoculated seedlings and determined for isolates from seedlings planted in the field. Identification was confirmed by species-specific PCR. To our knowledge, this is the first report of *P. capsici* infecting Fraser fir. This study suggests that planting Fraser fir in fields infested with *P. capsici* could result in infection.

Cross comparison of soybean gene expression upon infection by pathogens and the symbiont *Bradyrhizobium japonicum*

O. E. RADWAN (2), O. Zernova (2), V. V. Lozovaya (2), S. J. Clough (1)
(1) Dept. of Crop Sciences, USDA-ARS Soybean/Maize Germplasm, Pathology and Genetics Research Unit, University of IL, Urbana, IL 61801; (2) Dept. of Crop Sciences, University of Illinois, Urbana, IL
Phytopathology 98:S129

Pseudomonas syringae pv. *glycinae* and *Fusarium virguliforme* (formally known as *F. solani* f. sp. *glycines*) are examples of bacterial and fungal pathogens which attack soybean and can cause significant damage under environmental conditions favorable to the pathogens. *Bradyrhizobium japonicum* is a nitrogen-fixing bacterium which develops a symbiotic relationship with soybean roots. Using genomic approaches, such as microarrays and real time PCR technologies, we are detecting global changes in gene expression during these interactions. Preliminary results from the cross comparison of gene expression data from these different interactions indicate that most genes show similar expression patterns in *P. syringae* challenged leaves as in *F. virguliforme* challenged roots, suggesting that many defense responses are shared between these two very different tissues, and two very different pathogens. For example, pathogenesis related proteins and WRKY transcription factors were clearly up regulated in the case of *P. syringae* incompatible versus compatible interactions, and were also up in roots challenged with *F. virguliforme*, whereas these genes were down regulated in the *B. japonicum*-soybean interaction. These results suggested that plants employ many similar defenses and signaling components regardless of tissue type, and that B. japonicum suppresses their expression while establishing a symbiotic relationship. Functional analysis of defense and signaling components that are shared, as well as a few that differ during pathogenic and symbiotic interactions, is currently being conducted.

Epidemiological significance of *C. gloeosporioides* infestation of nursery plants on crown rot of strawberry

M. RAHMAN (1), F. Louws (1)
(1) NC State University, Raleigh, NC, USA
Phytopathology 98:S129

Anthracnose crown rot caused by *Colletotrichum gloeosporioides* caused significant losses to strawberry (*Fragaria* × *ananassa*) nursery and fruit growers in the southeast U.S. in recent years. Nonsymptomatic infested plants from nurseries are the major source of inoculum in the fruiting field. The epidemiological significance of plant infestation in the nursery on plant mortality and berry yield in fruiting fields was studied. A nursery experiment was established in May 2007 with registered Chandler plants with 4 treatments arranged in an RCBD in 4 replicates. Treatments comprised 0, 5, 10 and 25% of mother plants inoculated with 5 × 10^6 *C. gloeosporioides* spores/ml. Evaluation of quiescent infections on leaves using a paraquat dip method indicated a strong correlation for both incidence (R^2 = 0.99) and severity (R^2 = 0.92) with % inoculated mother plants at 40 days after inoculation, which decreased over time. Plant counts from random quadrants indicated daughter plant production was negatively and significantly (P < 0.0001) impacted by *C. gloeosporioides*. Dispersal gradients of inoculum from mother to daughter plants had a good fit using the empirical power law model. Infested tips led to 35% mortality in plug trays, indicating the enormous risk of producing plug plants with infested tips. Fall-set daughter plants in the fruiting field died at a steady rate. Mortality rates slowed down during winter, except in the 25% treatment where mortality of plants continued through the winter. The effect of nursery plant infestation on plant biomass, root dry weight, plant vigor and marketable berry yield was documented. Dipping infested plants in selected fungicides before fall planting decreased crown rot incidence from 42.75% to 13.33% within the first 4 months of observation.

Anthracnose resistance in strawberry genotypes for plasticulture systems in the Southeast

M. Rahman (1), F. LOUWS (1)
(1) NC State University, Raleigh, NC, USA
Phytopathology 98:S129

Anthracnose fruit rot (AFR) caused by *Colletotrichum acutatum* is the most devastating disease of strawberry (Fragaria × ananassa) in plasticulture systems in the Southeast U.S. due to rain splash-driven spore dispersal on plastic mulch under warmer weather conditions. Host resistance offers the best option to limit crop losses since it is difficult to manage and prevent the introduction of latent infections on planting stock that leads to AFR outbreaks in fruiting fields. To evaluate levels of anthracnose resistance and unravel mechanisms, we evaluated 14 strawberry cultivars and breeding lines from the NC State strawberry breeding program. Field inoculation of plants with three representative C. acutatum isolates indicated that the commercially standard cultivar Chandler was highly susceptible, with an average fruit rot incidence of over 70%. In contrast, breeding lines such as NCC 99-27 and NCC02-63 were moderately to highly resistant with 18.32% and 4.86% incidence, respectively, and showed superior total yields. Sequencing of a glutamine synthetase gene intron of 12 C. acutatum isolates collected from fruit and latent infections on leaves from NC strawberry epidemics demonstrated a high level of homogeneity/clonality in the population and helped in selecting isolates for this study. *In vitro* assessment of anthracnose severity on detached fruit was highly correlated (R^2 = 0.81, $P \leq$ 0.003) with field incidence. Enumeration of spores on individual AFR lesions revealed significant difference among genotypes and thus may be a component of resistance with a lower potential of secondary spore production. Latent infection incidence on whole plants inoculated with 5 × 10^6 spores/ml in phytotron studies was not correlated (R^2 = 0.35, ns) with field AFR incidence, indicating different mechanisms may be operative for resistance to latent infections. Total phenolic content in berries of different genotypes did not have a strong correlation with resistance. Estimation of individual phenolic compounds and defense enzymes are in progress.

Role of garden centers and retail nurseries in spreading citrus huanglongbing disease

C. RAMADUGU (3), K. L. Manjunath (2), C. Ramos (5), S. Halbert (1), S. Webb (4), R. F. Lee (2)
(1) DPI, Gainesville, FL, USA; (2) USDA-ARS, Riverside, CA, USA; (3) University of California, Riverside, CA, USA; (4) University of Florida, Gainesville, FL, USA; (5) University of Panama, Panama
Phytopathology 98:S129

Huanglongbing (HLB) or citrus greening disease was first reported from Florida in 2005. The psyllid vector, *Diaphorina citri* has been present in Florida since 1998. Psyllid samples were collected from a large number of garden centers and retail nurseries over a period of about three years. These psyllids were analyzed for the presence of HLB-associated bacterium, *Candidatus* Liberibacter asiaticus. Results showed the presence of the bacterium in psyllids collected from several retail outlets, in some cases well before the establishment of the disease in the area. Plants from these outlets are not only sold throughout the state, but possibly find their way into other citrus growing states as well. In several cases repeat samples were also

positive for the presence of the bacterium. Under field conditions, the bacterium is known to thrive well in certain seasons only. Retail stores without temperature extremes and with plants having new growth flushes throughout the year would probably serve as an ideal environment for both psyllids and the bacteria. The study shows the importance of psyllid-free nursery retail stores as a part of disease management in locations where HLB is not yet established.

A new phytoplasm associated disease of chile peppers
J. J. RANDALL (2), P. Bosland (1), S. F. Hanson (2)
(1) Department of Plant and Environmental Science, NMSU, Las Cruces, NM, USA; (2) EPPWS, New Mexico State University, Las Cruces, NM, USA
Phytopathology 98:S130

Chile pepper producers in NM have been reporting the increasing occurrence of plants that fail to set fruit over the past several years. Other than failing to set fruit, phenotypes of affected plants varied. Occasionally, affected plants also displayed a mosaic chlorosis with leaf thickening and cupping consistent with virus infection. Close examination of these plants showed that the failure to set fruit was due to aberrant flower development. All affected plants displayed abnormally large green buds that failed to develop into flowers. These symptoms are similar to those reported for several phytoplasm caused diseases in other solanaceous crops such as Big Bud of Tomato. Light and electron microscopy showed phytoplasm like bodies distributed throughout the phloem tissue of affected plants while no similar objects were observed in healthy control plants. Polymerase chain reaction testing with phytoplasm specific primers 16S and 23S rDNA primers produced amplicons of the expected size from all symptomatic plants while no amplicons were recovered from symptomless plants. Ribosomal gene and ITS region sequences were most closely related to other phytoplasmas but differed significantly from previously described phytoplasmas, especially those reported to infect solanaceous crops. Together these data suggest that the failure to set fruit phenomenon recently observed in the Southwestern U.S. is a new disease of chile peppers caused by a novel phytoplasm.

Distribution and variability of a new chile pepper infecting phytoplasm
J. J. RANDALL (1), S. F. Hanson (1)
(1) EPPWS, New Mexico State University, Las Cruces, NM, USA
Phytopathology 98:S130

A new phytoplasm associated disease of chile peppers (capsicum annum) was recently characterized by our group. The disease causes a failure in fruit set and therefore has the potential to greatly decrease yields. Affected plants develop abnormally large buds that fail to develop to flowers and has thus been named “Brote Grande” (Spanish for Big bud) of pepper. Associated symptoms also include phylody and leaf thickening on affected plants. Stunting and chlorosis are also observed in some affected plants. The disease was initially described near the city of Las Cruces in Southern New Mexico. As part of this characterization, a reliable PCR based assay for amplification and sequencing of 16S-23S rDNA amplicons was developed. Here we report results of a survey across chile producing areas in New Mexico and Arizona where these primers were used to characterize phytoplasm sequences in symptomatic plants. The results of this survey show: 1) that this disease was present at low frequency in all chile production areas tested across the Southwestern U.S., 2) that there is very little variability in the pathogen across the region, 3) that double infections with the phytoplasm and Beet Curly top virus are common, and 4) that this double infection is associated with the stunted chlorotic phenotype sometimes observed with this disease.

Sunflower rust races in Manitoba, Canada
K. RASHID (1)
(1) Agriculture and Agri-Food Canada, Morden, MB, Canada
Phytopathology 98:S130

Sunflower rust caused by *Puccinia helianthi* Schwein. is a common disease affecting sunflower (*Hilianthus annuus* L.) worldwide. This disease is widely spread in the sunflower growing areas in North America and reduces the yield by up to 25% in addition to the reduction in quality of seed. This pathogen completes its life cycle on sunflower with a high frequence of the development of new races. Rust isolates were collected from all sunflower growing regions in Manitoba, Canada from 2003 to 2007, and the virulence of these isoltes were assessed on a ste of nine host differential sunflower genotypes under controlled growth room conditions. The rust race groups 300 and 700 were the most common in Manitoba. Race group 700 which includes the races 726, 776, and 777 proved to be more virulant than the race group 300 which includes the races 326, 334, and 336. However, the race group 700 is presently at a lower frequency than the group race 300 in Manitoba. Races 100 and 500 were at a low frequency in the rust population. Most commercial sunflower hybrids express various levels of resistance to race groups 100 and 500 but not to race groups 300 and 700.

Molecular characterizatioin of Wheat Eqlid mosaic virus
M. Rastegar (1), K. IZADPANAH (1), M. Masumi (1), M. Siampour (1), A. Zare (1), A. Afsharifar (1)
(1) Shiraz University
Phytopathology 98:S130

Wheat Eqlid mosaic virus (WEqMV) is a flexuous rod virus infecting several poaceous species in Eqlid, a wheat growing region in the Fars province of Iran. Electron microscopy and limited molecular studies have previously shown that this virus is a member of the family *Potyviridae*. In the present study, the complete genome of this virus was sequenced and analyzed. The genome consisted of 9636 nucleotides including 5′ and 3′ UTR of 137 and 172 nucleotides, respectively, and a single open reading frame coding a polyprotein of 3109 amino acids. Phylogenetically, WEqMV was closest to Wheat streak mosaic virus, with a similarity of 56.8% and 50.7% at nucleotide and amino acid levels, respectively in the total genome. It was less similar to other tritimoviruses. The proteinase cleavage sites of the polyprotein in WEqMV were also very similar to those of other tritimoviruses. Based on These data, WEqMV is regarded as a new member of the genus *Tritimovirus* in the family *Potyviridae*. This study was supported by funds from Centers of Excellence and TWAS-IC.

Relief of abiotic stress in corn by DAPG-producing *Pseudomonas fluorescens* strain Wood1R under acidic soil conditions
R. E. RAUDALES (1), E. Stone (1), B. B. McSpadden Gardener (1)
(1) Plant Pathology, The Ohio State University, Wooster, OH, USA
Phytopathology 98:S130

Flurorescent *Pseudomonas* spp. that produce the 2, 4-diacetylphloroglucinol (DAPG) are well known for their capacity to suppress diverse soilborne pathogens. However, little is known about their potential to improve plant health under abiotic stress conditions (i.e. low pH soils). Our objective was to determine if *P. fluorescens* strain Wood1R had the ability to improve plant health and productivity of corn in soils with a pH of 4.7. Two field and three greenhouse experiments were established in which corn seeds were treated with Wood1R. The plants were evaluated for stand, height, leaf area covered with lesions, and, for the field experiments, yields. Nutrient uptake in leaves was also assessed. In the field, plants treated with Wood1R were taller ($P < 0.05$), had less leaf area covered with stress-related lesions ($P < 0.1$) and had greater yields ($P < 0.1$) compared to the negative control. Two of the greenhouse experiments showed reduction in the foliar area covered with lesions ($P < 0.05$) when treated with Wood1R. No differences in stand were observed for any experiment, indicating that pathogen suppression was not a factor. Foliar levels of P and Mg were significantly greater ($P < 0.1$) in Wood 1R-treated plants in two of four experiments. These data indicate that the plant health promotion conferred by DAPG-producing pseudomonads may be related to alterations in soil nutrient uptake by plants when grown in low pH soils.

Effect of isolate, environment and a defeated R-gene ($R_{Pi\text{-}ber}$) on quantitative resistance of potato to late blight
G. RAUSCHER (4), H. Mayton (3), M. Bonierbale (1), I. Simko (6), C. Smart (2), N. Grunwald (5), W. Fry (3)
(1) Centro Internacional de la Papa, Lima, Peru; (2) Cornell University, Geneva, NY; (3) Cornell University, Ithaca, NY; (4) Cornell University, Ithaca, NY / USDA-ARS, Salinas, CA, USA; (5) USDA-ARS, Corvallis, OR; (6) USDA-ARS, Salinas, CA, USA
Phytopathology 98:S130

Phytophthora infestans, the causal agent of late blight is the most aggressive and costly pathogen affecting potato production worldwide. Genetic resistance is gaining importance as the pathogen continues to develop resistance to fungicides. However, little is known about the stability and specificity of quantitative resistance including the effects of defeated major resistance genes. In this study we analyzed resistance to *P. infestans* in two reciprocal inter-specific backcrosses of *Solanum tuberosum* and *S. berthaultii*. Analyses were done with five different strains of *P. infestans*. The experiments were conducted in different environments including field, plastic house and mist chamber. Inoculations were done by applying a suspension of sporangia to each plant, the percentage of the total tissue affected was scored every two or three days, and the area under the disease progress curve (AUDPC) was calculated. Phenotypic data was added as trait data for QTL mapping using MapManager QTX. We found a significant and lasting effect located in the same genetic position as a defeated R-gene ($R_{Pi\text{-}ber}$) in interactions with compatible *P. infestans* isolates. Our results also show quantitative resistance loci (QRLs) with a general effect (isolate nonspecific) in chromosomes III, V and X. Minor QRLs with isolate specificity were found scattered around the genome. These results are consistent with a lingering effect of a defeated R-gene.

Enhancement of pathogenicity of *Burkholderia andropogonis* isolated from citrus by pthA or pthB from *Xanthomonas citri*

G. D. REBELLO (1), D. W. Gabriel (1)
(1) University of Florida, Gainesville, FL, USA
Phytopathology 98:S131

Burkholderia andropogonis (Ba) causes a variety of leaf spot diseases world wide on multiple hosts, and was first identified as a citrus disease agent in 2007. *Xanthomonas citri*, the causal agent of citrus canker, can horizontally transfer *pthB*, a *pthA* family (type III effector) gene to other bacteria *in planta* on a self mobilizing plasmid (El Yacoubi et al., 2007). Since Florida's canker eradication program has been suspended, there is greatly increased opportunity for horizontal gene transfer involving *X. citri* in Florida. Electroporation of *pthA* into Ba increased pathogenicity, including intensification of chlorosis and necrosis on grapefruit, lime, sweet orange and tangerine leaves. However, Ba transformants carrying *pthA* or *pthB* grew at least one log better than wild type in all citrus varieties tested, suggesting that the *pthA* and *pthB* effectors can suppress citrus defenses. Surprisingly, no transformant caused symptoms typical of citrus canker, indicating inefficient, and still hypothetical, type III transfer. No type III system has been described in Ba, but we have amplified apparent homologs of type III system genes *sctS* and *sctT* from Ba.

Detection and management of downy mildew of blackberry caused by *Peronospora sparsa* in Michoacan, Mexico

A. REBOLLAR-ALVITER (2), H. V. Silva-Rojas (1), L. X. Zelaya-Molina (1)
(1) Colegio de Postgraduado/Produccion de Semillas, Montecillo, Mexico, 56230; (2) Universidad Autonoma Chapingo/Centro Regional Morelia, Morelia Michoacan, Mexico, 58170
Phytopathology 98:S131

Dryberry is one of the most important limiting factors of blackberry industry in Michoacan, Mexico. During the raining season, growers have lost up to 100% of their production due to this disease, mainly by the lack of knowledge about the epidemiology, control, and early detection. The objectives of this research were to confirm the presence of *Peronospora sparsa* associated to dryberry in Michoacan, and to evaluate fungicide programs to control the disease in two different regions during the raining and dry season of 2007 and 2008. Total DNA from symptomatic fruits, symptomatic and asymptomatic leaves were extracted, and analyzed for the presence of *P. sparsa*. A nested PCR using PS3/PS1 primer pair followed by PR3/PR4 was used to amplify the ITS region. The nucleotide sequence analysis of an amplicon of 500 bp from all samples confirmed the presence of *P. sparsa* (Genbank-NCBI accession number: EU369694). Fungicide trials during the raining season showed that all treatments beginning with potassium phosphite every 10 to 14 days reduced the dryberry incidence by more that 50% compared to the control (70% incidence). During the dry season, disease incidence was very low, but those treatments including potassium phosphite did not have symptomatic fruits or leaves. Early detection of *P. sparsa* by PCR and treatments including potassium phosphite could help reducing the disease incidence on blackberry in Mexico.

Shoot blight and anthracnose of blackberries in Mexico is caused by *Glomerella cingulata*

A. REBOLLAR-ALVITER (2), H. V. Silva-Rojas (1), L. X. Zelaya-Molina (1)
(1) Colegio de Postgraduado/Produccion de Semillas, Montecillo, Mexico, 56230; (2) Universidad Autonoma Chapingo/Centro Regional Morelia, Morelia Michoacan, Mexico, 58170
Phytopathology 98:S131

Blackberry cultivated area in Mexico is near 6000 ha, and 80% of this area is located in Michoacan State. Shoot blight and anthracnose is a very common disease during the raining season, affecting seriously the production of this fruit. The main objectives of this research were to identify the causal agent of shoot blight and anthracnose, and to do *in vitro* testing of some commonly used fungicides for controlling this disease. Samples of blackberry plants showing shoot blight and anthracnose were recorded and used to do isolations on PDA medium. Pathogenicity test were conducted by spraying the shoots with a suspension of 10^6 conidia/ml obtained from an isolate producing abundant sporulation. Inoculated shoots were incubated in a moist chamber for 48 h at room temperature. The first symptoms appeared three days post-inoculation on younger leaves, and six days later, the whole shoot was completely blighted. To identify the causal agent, DNA was extracted from mycelia of 11 isolates, and ITS region was amplified and sequenced using ITS5/NL4 primer pair. The phylogenetic analysis identified the isolates as *Glomerella cingulata* (Genbank accession numbers: EU358943-EU358953). *In vitro* fungicide tests showed good to excellent activity of captan, mancozeb, copper sulfate, copper oxychloride, phosphite and a sanitizing agent at commercial rates. These results report the presence of a new pathogen on blackberry in Mexico, and the activity of some fungicides for disease management.

RetS is a *Pseudomonas syringae* B728a hybrid sensor kinase that controls swarming, regulates expression of the type VI secretion system, and contributes to colonization of bean leaves

A. R. RECORDS (1), D. C. Gross (1)
(1) Texas A&M University, Dept. of Plant Pathology & Microbiology, College Station, TX
Phytopathology 98:S131

Bacterial pathogens utilize two-component systems (TCSs) to respond to environmental changes and to regulate the expression of virulence traits. A typical bacterial TCS is composed of a histidine kinase (HK) and a response regulator (RR), which is phosphorylated by the HK in response to an environmental signal. *Pseudomonas syringae* pv. *syringae* strain B728a (*Pss* B728a) is a cosmopolitan pathogen that is highly adapted to life on the surface of bean leaves, but has the versatility to invade stomata under appropriate environmental conditions. The *Pss* B728a genome is predicted to encode 68 HKs and 93 RRs, which contribute to the adaptation of this bacterium to plant and non-plant environments. RetS is a unique hybrid sensor kinase with a modular structure consisting of an HK followed by two RR domains. RetS is known to regulate critical virulence pathways for the human pathogen *Pseudomonas aeruginosa*, including the type III secretion system (T3SS), the newly-discovered type VI secretion system (T6SS), biofilm formation, and swarming motility. We have identified a *retS* homolog in the genome of *Pss* B728a. Like its *P. aeruginosa* counterpart, *Pss* B728a RetS also controls the T6SS and swarming motility. In addition, we have found that *Pss* B728a RetS influences colonization of bean leaves. We are currently characterizing a functional T6SS encoded in the genome of *Pss* B728a, and we are investigating the role of the T6SS in *Pss* B728a pathogenic fitness. RetS has a negative effect on the T6SS; in-culture type VI secretion by B728a is markedly enhanced by a mutation in *retS*. RetS is clearly an important global regulator, and our long-term goals include characterizing the RetS regulon at the whole-genome level.

Studies in sour orange and C-22 rootstocks challenged with the nematode, *Tylenchulus semipenitrans* and the fungus, *Phytophthora parasitica*

V. P. Reddy (1), M. Kunta (1), J. V. Da Graca (1), S. Nelson (2), M. SKARIA (1)
(1) Texas A&M University-Kingsville Citrus Center, Weslaco, TX, USA; (2) Texas A&M University-Kingsville, Kingsville, TX, USA
Phytopathology 98:S131

The aim of this study is to investigate the reactions of two citrus rootstocks, C-22 (a trifoliate hybrid) and sour orange when challenged with the nematode, *Tylenchulus semipenitrans* and the fungus, *Phytophthora parasitica*. Quantitative studies include: number of female nematodes, larvae, and eggs on root and juveniles in soil, correlated to root mass density. The resistance of citrus rootstocks to nematode and *Phytophthora* infections will be evaluated with length of lesions and reductions in shoot length, root mass and shoot mass. From inoculation experiments, we found that in both the rootstocks, 10 h after inoculation, zoospores of *Phytophthora* successfully penetrated into the roots. Both rootstocks, when inoculated with juvenile nematodes, resulted in successful penetration within 12 d after inoculation. Total RNA was extracted from both the inoculated and the non-inoculated root samples and double stranded complementary DNA (ds cDNA) was synthesized. This ds cDNA will be used as a template in amplified fragment length polymorphism (AFLP) to study the differences in gene expression.

Genetic diversity in *Sclerotium rolfsii* infecting sugar beet in Mediterranean environments

E. REMESAL (3), B. B. Landa (3), R. M. Jimenez-Diaz (2), M. M. Jimenez-Gasco (1), J. A. Navas-Cortes (3)
(1) Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA; (2) Instituto de Agricultura Sostenible, CSIC and ETSIAM, Universidad de Córdoba, Campus Rabanales, 14071 Córdoba, SPAIN; (3) Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain
Phytopathology 98:S131

Root rot caused by *Sclerotium rolfsii* has been previously reported in areas with Mediterranean climate but the genetic structure of the population in these areas is not well understood. Among 277 single isolates of *S. rolfsii* representing 16 localities from Chile (seven isolates), Italy (10 isolates), Portugal (62 isolates) and Spain (198 isolates), we analyzed mycelial compatibility groups (MCGs) based on the antagonism zone between incompatible mycelia of paired isolates. Twelve MCGs were identified, seven of them comprised of only one isolate; with two major MCGs present in most of the sampled locations. Individual fields contained up to two MCG. The

extent of genetic diversity among MCGs was studied by using restriction fragment length polymorphism (RFLP) patterns of the ITS of nuclear rDNA region (RFLP-ITS) digested with *Alu*I, *Hpa*II, *Rsa*I and *Mbo*I and by phylogenetic analysis from the second largest subunit of RNA polymerase II and the translation elongation factor genes. Based on RFLP-ITS analysis three groups could be distinguished, two of them subspecific within *S. rolfsii* and one in *S. delphinii*. Furthermore, for some MCGs, the total sum of digested fragment sizes exceeded the undigested PCR product. This suggests the presence of two distinct nuclear types, i.e., a possible heterokaryotic. Experiments are in progress to determine the number and types of nuclei per cell. Phylogenetic analysis could not identify discernible relationships among the various MCGs, but to some degree the groups identified were correlated with geographic origin of isolates and could clearly distinguish between *S. rolfsii* and *S. delphinii*.

Inoculation by antagonistic bacteria of slow-filtration unit for soilless cultures: Consequences on microbial communities colonizing the nutrient solutions

D. Renault (3), F. Deniel (3), S. Maurice (3), J. Godon (1), G. Barbier (3), P. REY (2)
(1) INRA, Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, 11100 Narbonne, France; (2) INRA, UMR 1065 Santé Végétale, Université de Bordeaux, ENITAB, 1 cours du Général de Gaule, 33135 Gradignan, France; (3) Laboratoire de Biodiversité et Ecologie Microbienne, ESMISAB, Université de Bretagne Occidentale, 29280 Plouzané, France
Phytopathology 98:S132

In soilless culture, slow filtration is used to eliminate pathogenic agents from nutrient solutions of plants. The present work was conducted over a whole cultural season (7 months), two filters were amended with bacteria having suppressive traits: *P. putida* or *B. cereus*-strains (P-filter and B-filter, respectively), a third filter was a control (C-filter). Biological activation of filter unit through bacterial amendment very significantly enhance filter effectiveness against *Pythium* spp. and *Fusarium oxysporum*; however, numerous bacteria (around 10^3-10^4 cfu.mL^{-1}) were detected in the effluent solutions. The community level physiological profiles (CLPPs) indicated a temporal shift of bacterial populations, the metabolism of nutrient-solutions originally oriented towards carbohydrates progressively shifted towards degradation of specific amino acids and carboxylic acids. SSCP fingerprintings showed that a shift between influent and effluent solutions of slow-filters occurred. In addition, effluent bacterial communities were different from influent ones when slow-filters were bacteria-amended (P and B filters). In comparison to influent, 16S rDNA sequencing revealed that phylotypes diversity was lower in the effluent from bacteria-amended filters, but no reduction was observed in the effluent from the control filter. The biocontrol agent potential of inoculated-bacterial strains to optimize slow filtration process and to promote suppressive potential of nutrient solution is discussed.

Root growth response to application and overexpression of *Heterodera glycines* CLE peptides

A. REPLOGLE (2), J. Wang (2), X. Wang (3), E. L. Davis (1), M. G. Mitchum (2)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA; (2) Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO, USA; (3) USDA-ARS, Biological IPM Research Unit and Dept. of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY
Phytopathology 98:S132

Plant CLAVATA3/ESR(CLE)-like peptides have been shown to be involved with several aspects of plant development including maintenance of stem cell pools in the root meristem. Interestingly, parasitism genes, *HgCLE-1* and *HgCLE-2*, encoding secreted CLE-like peptides are expressed in the dorsal esophageal gland cell of the soybean cyst nematode (SCN), *Heterodera glycines*, during syncytium induction and maintenance in host roots. HgCLE-1 and HgCLE-2, differ only in a variable domain N-terminal of the conserved CLE motif. Deletion of the CLE motif abolishes function in overexpression studies, and exogenous application of a synthetic dodecapeptide corresponding to the conserved CLE motif of HgCLEs is sufficient to induce a short root phenotype when applied to Arabidopsis roots. Despite their identical CLE motifs, constitutive overexpression of *HgCLE-1* but not *HgCLE-2* in Arabidopsis, a non-host for SCN induced root meristem defects similar to that of overexpression of plant *CLEs*. In constrast, when overexpressed in soybean hairy roots, a host for SCN, both *HgCLE-1* and *HgCLE-2* caused premature termination of primary root growth. Cell identity marker lines combined with confocal microscopy are being used to assess affects of nematode CLE peptides on root growth. These data suggest that there may be host-specific control of nematode CLE peptide recognition. This indicates that the evolution of nematode *CLE* genes could explain one of the underlying mechanisms driving the specific adaptation of cyst nematodes to parasitize particular host plant species.

Epistatic and quantitative resistance loci against philippine bacterial blight races 6 and 9 for resistance breeding and crop management

J. D. REY (2), C. M. Vera Cruz (2), M. S. Mendioro (1), D. J. Mackill (2), A. A. Barrion (1), J. Xu (2), Z. Li (2)
(1) IBS, UPLB, Philippines; (2) IRRI, Makati City, Philippines
Phytopathology 98:S132

Epistatic and quantitative resistance loci (QRL) for the disease reaction of two BC_2 populations were detected using both artificial inoculation of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) under screenhouse conditions and simple sequence repeats (SSR) analysis. These drought tolerant- (DT) and grain quality- (GQ) selected introgression lines (ILs) exhibited resistance to Philippine Xoo Races 6 and 9. A total of 182 polymorphic SSR markers were used to genotype the three parents (IR64 and Teqing as recurrent parents, Binam as donor) and 175 ILs. The parents were susceptible to Race 6 and resistant to Race 9. Three ILs in the IR64/Binam population exhibited complete resistance (CR) to Race 6, which was associated with six markers located in five chromosomes. The absence of one locus RM411 led to a susceptible reaction, while the presence or absence of all six loci in different combinations led to resistance or susceptibility. These phenotypic reactions could be due to epistasis suggesting a common pathway for resistance. Twenty-eight DT and GQ ILs exhibited CR to Race 9 with only one new putative QRL associated. In the Teqing/Binam population, partial resistance (PR) to race 6 was associated with 4 new marker loci in three chromosomes, two of the loci can be identified as putative main effects QRLs and the other two as minor effects QRLs. Seventy-three ILs showed CR to Race 9, one new putative QRL associated with CR was identified in this study and two putative QTLs conferring susceptibility. The results demonstrated multiple interactions in the rice-Xoo pathosystem which could lead to CR, PR, or susceptibility. These results can be used for broad-spectrum resistance breeding in rice against Races 6 and 9, that could lead to better crop management.

Cellulase activity and microbiology of cultural systems for Phytophthora root rot control in Fraser fir

B. S. RICHTER (2), D. M. Benson (2), K. L. Ivors (1)
(1) NC State University, Fletcher, NC, USA; (2) NC State University, Raleigh, NC, USA
Phytopathology 98:S132

Phytophthora root rot of Fraser fir, caused by several *Phytophthora* spp., is a severe problem in Christmas tree production. As fungicides and host resistance are ineffective in long term disease control, cultural control systems are being tested at five naturally infested field sites. Treatments include wood chips (WC), wood chips plus compost (WCC), or pine bark (PB) as raised beds, and compost or sulfur tilled into soil. Microbial populations and activity were characterized by dilution plating, fluorescein diacetate hydrolysis (FDA), and cellulase enzyme activity. Bacterial and fungal counts were generally higher in mulches than in soil, while counts of cellulose degraders were less consistent and rarely showed significant differences. Cellulase activity was significantly higher in mulch than soil. In soil and upper mulch, there was a correlation between cellulose degrader counts and cellulase activity, but none was observed in lower mulch, where activity was usually highest. Cellulase activity was correlated with total microbial activity (FDA) in soil but not in mulch. Tree survival was significantly higher in mulched plots at two (alpha = 0.05) or three (alpha = 0.10) of five sites; WC and WCC had higher tree survival than control or compost plots at all sites. One year after planting, survival was positively correlated with cellulase activity in lower mulch ($r = 0.66$, $P = 0.07$) and total activity in soil ($r = 0.67$, $P = 0.006$).

Antagonism between biofumigation and biocontrol in the soil

E. RIGA (1), D. Henderson (3), W. Snyder (2), R. Ramirez (2)
(1) Washington State University, Prosser, WA; (2) Washington State University, Pullman, WA; (3) Washington State University, WA
Phytopathology 98:S132

Synthetic fumigants have traditionally been used to control plant-parasitic nematodes and other soil-borne pests, but the negative environmental effects of these pesticides have sparked a search for more benign replacements. One alternative is the use of mustard green manures or seed meal high in glucosinolate compounds. However, the non-target impacts of these bio-fumigants are unclear. In a large-scale, multi-year field experiment, we examined the effectiveness of applications of *Brassica carinata* seed meal, to potato crops before planting, in controlling the plant-parasitic Columbia root knot nematode (*Meloidogyne chitwoodi*). We also measured disruptive effects of mustard seed meal application on the biological control exerted by beneficial *Steinernema* spp. nematodes on the root knot nematodes and a key insect pest, the Colorado potato beetle (*Leptinotarsa decemlineata*). Mustard seed meal applications were conducted as a fully-factorial cross with

inundative releases of the nematodes *Steinernema feltiae* or *S. riobrave*. Singly, both mustard and beneficial-nematode application reduced root knot nematode damage to potato tubers and increased marketable tuber yields. However, there was a negative interaction between the two bio-agents such that the combination of these two control options did not further improve suppression of plant-parasitic nematodes. Thus, mustard seed meal applications toxic to the target pest, the root knot nematodes, also disrupted the ability of *Steinernema* spp. to act as a biocontrol agent. *Steinernema* spp. also attacked Colorado potato beetles pupating in the soil. Field and laboratory experiments indicated some disruption of this biological control effect on the beetles following biofumigation. However, potato beetles were less-willing to lay eggs on potato plants grown in mustard amended soil, suggesting a counteracting benefit of mustard application. Our results demonstrate that mustard bio-fumigants have complex non-target effects on the potato food web, with both negative and positive implications for crop production.

Chemotaxis of Phytophthora zoospores to soybean roots is altered by RNAi silencing of isoflavone biosynthesis

K. J. RIGGS (1), T. L. Graham (1)
(1) Ohio State University, Plant Pathology, Columbus, OH, USA
Phytopathology 98:S133

Phytophthora sojae zoospores navigate to the roots of soybean (*Glycine max*) through chemoattraction to substances exuded by the roots. Although the isoflavones, daidzein and genistein, may be primary chemoattractants for *P. sojae*, their roles relative to other potential attractants are unknown. *Agrobacterium rhizogenes* was used to initiate and transform soybean hairy roots with RNAi constructs to silence genes for two different enzymes for isoflavone biosynthesis, isoflavone synthase (IFS) and chalcone reductase (CHR). Excised roots (2 cm long) were placed in the wells of concave microscope slides. *P. sojae* zoospores were introduced, submersing the roots fully. Zoospore movements were monitored until the zoospores encysted. The numbers of zoospores attached to the roots were then recorded, noting the population of zoospores in each of five designated root sections. In control roots the largest number of zoospores attached to a specific zone corresponding to the zone of elongation. In roots containing neither genistein nor daidzein, due to IFS silencing, the zoospores did not attach to the normal root zone, but instead attached to the root cap. In contrast, the number of zoospores attracted to CHR-silenced roots was three times that of control roots, but zoospores were attached along the entire root. Taken together, these results suggest that genistein is a strong chemoattractant for *P. sojae*, but that daidzein may be responsible for the specific targeting of zoospores to the correct root zone. Continued study of mechanisms underlying *P. sojae* zoospore chemotaxis could lead to improvements of disease resistance in soybean and related legumes.

Source of more than 60 years of chemical disease-control data: The publication 'Fungicide and Nematicide Tests', 1945–2006

D. F. RITCHIE (1), K. S. Yoder (3), D. S. Egel (2)
(1) N.C. State University, Raleigh, NC, USA; (2) Purdue University, SW Purdue Ag. Center, Vincennes, IN, USA; (3) VA Tech Agric. Res. & Ext. Center, Winchester, VA, USA
Phytopathology 98:S133

Synthetic, organic fungicides were introduced in the mid-1930s. These revolutionized plant disease control, started a wave of product evaluations, and the compilation and exchange of disease-control data. The Potomac Division of APS in 1945, appointed a committee to "undertake the collecting, classifying, summarizing and mimeographing disease-control data generously contributed by pathologists throughout the United State and Canada" (F&N TESTS, vol. 50). In 1960, the word "Nematocide" was included in the title and changed to "Nematicide" in 1969. The 61 volumes of F&N TESTS have been published as supplements to the USDA Plant Disease Reporter, Agricultural Chemicals, privately, and by APS Press. Since the 2000 edition (volume 55), the reports are available electronically in PDF-format at the APS website. In 2007, F&N TESTS and Biological and Cultural Tests were combined into Plant Disease Management Reports. From 1961–2006, approximately 16,000 reports were compiled and published. The initiation, continuation, and growth of F&N TESTS resulted from countless hours contributed by numerous APS members. For most of its existence, F&N TESTS has been guided by an editor, section editors, and the APS New Fungicide and Nematicide Data Committee members. Information in pictorial, graphic, and narrative format describes some of these contributions, the history, and processes used to produce F&N TESTS. This publication may be the single, richest source for information of the development of applied chemical plant-disease control and the individuals and industries that contributed.

High tunnels and grafting for disease management in organic tomato production

C. L. RIVARD (2), F. J. Louws (2), M. M. Peet (1), S. O'Connell (1)
(1) North Carolina State University, Dept. of Horticulture, Raleigh, NC, USA; (2) North Carolina State University, Dept. of Plant Pathology, Raleigh, NC, USA
Phytopathology 98:S133

High tunnels are gaining popularity for organic and conventional tomato growers as they may provide season extension, reduced foliar disease incidence (particularly against early blight and *Septoria*), and increased fruit quality. However, crop rotation is often compromised, and soilborne disease can be prevalent in these systems. A research program was initiated to evaluate commercially-available tomato rootstocks capable of reducing soilborne disease incidence and increasing yield under tunnel and field conditions. Soilborne diseases such as bacterial wilt, root-knot nematode, and southern stem blight were effectively managed utilizing various rootstocks in infested field and tunnel soils ($P < 0.05$). An experiment was established at the Center for Environmental Farming Systems (Goldsboro, NC) to compare grafted and non-grafted plants within field and high tunnel production. The systems comparison showed that total fruit yield was higher in the tunnels ($P = 0.01$). Insect damage and tomato spotted wilt virus incidence was higher in the field ($P < 0.05$). In contrast, fruit cracking and catfacing incidence was higher in the tunnel system ($P < 0.05$). The main effect of grafting showed that yields were increased when 'Maxifort' rootstock was used, even under little disease pressure from soilborne pathogens ($P < 0.01$). High tunnels and grafting with resistant rootstock offer complementary roles in an integrated pest management approach for tomato.

Effects of sanitation with a bleach solution on daylily and hosta growth

E. ROBAYO CAMACHO (1), S. N. Jeffers (1)
(1) Department of Entomology, Soils, and Plant Sciences; Clemson University; Clemson, SC, USA
Phytopathology 98:S133

Daylilies (*Hemerocallis* spp.) and hostas (*Hosta* spp.) are two of the most popular and economically important herbaceous perennial plants produced in nurseries and planted in landscapes. Both are propagated vegetatively--often by planting dormant rooted crowns. However, soilborne plant pathogens can be present on propagation stock at the time of planting, and these propagules can serve as primary inoculum to initiate disease. Therefore, sanitation of propagation stock prior to planting can be an effective and economical disease management strategy if treatments have no deleterious effects on plant growth. In this study, we evaluated the effects of 10% household bleach solution on growth of Stella d'Oro daylilies and three cultivars of hostas--Albo Marginata, Aureo Marginata, and Wide Brim. Dormant crowns were soaked for 0, 1, 5, and 10 min in bleach solution and then rinsed thoroughly in tap water. Individual crowns were planted in a peat-based container mix in 3-liter pots, and pots were placed outside and randomized. Plants were watered as needed and allowed to grow for 40 to 50 days. Then, foliage from all plants was harvested and weighed; the numbers of flower stalks, flower buds, and flowers on daylily plants were counted at 26 or 28 days. Two trials were conducted with daylilies, and three trials were conducted with hostas. Based on analyses of variance, soaking dormant crowns of daylilies and hostas in 10% bleach solution for 1 to 10 min had no significant effect on the plant growth parameters measured. Consequently, a pre-plant soak in 10% bleach solution could be an effective and economical disease management practice for these two herbaceous perennial crops.

Global analyses of defence gene expression in a model tomato-Verticillium pathosystem

J. E. ROBB (1), C. D. Castroverde (1), A. C. Kent (1), R. N. Nazar (1)
(1) University of Guelph, Guelph, Ontario, Canada
Phytopathology 98:S133

Relatively little is known about the molecular mechanisms utilized by plants to defend themselves against fungal vascular pathogens. Here, we have used a model tomato-Verticillium pathosystem to compare the global expression of genes in compatible and incompatible interactions. Seedlings of Craigella tomatoes, susceptible or resistant to Verticillium dahliae race 1, were infected by root dipping in conidial suspension. Initially microarray analyses were carried out at one critical time point (10 days post-inoculation) using a commercially available DNA chip (TOM 1). At least 45 genes were significantly down-regulated in resistant relative to susceptible reactions and 88 genes were up-regulated. Of special note was a group of 14 genes forming a distinct scatter plot cluster. For comparison, gene expression in each interaction relative to uninfected control plants also was examined. Based on this and a previous study of a tolerant interaction, 267 genes were selected to make a custom chip for Verticillium-related genes which was used for more detailed comparisons during 4 to 15 days post-inoculation. Three main patterns of change were observed and confirmed by RT-PCR. One group comprised genes which were up-regulated in the resistant interaction relative to the susceptible; their expression correlated with the fungal colonization cycle. In the other two groups the genes were down-regulated in resistant

plants and the pattern correlated with various aspects of pathogenesis. Supported by the Natural Sciences and Engineering Council of Canada.

Status of viruses causing symptoms in pumpkin and watermelon in Puerto Rico

J. V. RODRIGUES (2), L. Wessel-Beaver (1)
(1) University of Puerto Rico, Department of Agronomy & Soils, Mayaguez, PR; (2) University of Puerto Rico, Department of Crop Protection, Mayaguez, PR

Phytopathology 98:S134

Cucurbits provide important basic ingredients for the Caribbean diet. Pumpkins are the second most important vegetable crop in terms of revenue generated in Puerto Rico. Virus and severe virus vector outbreaks are a frequent and major cause of low yields and phytosanitary limitations to growing cucurbits in Puerto Rico. A survey was conducted to assess the types and prevalence of viruses infecting cucurbits. A total of eighty-seven cucurbit plants (mainly pumpkin and watermelon) showing virus-like symptoms were sampled in ten municipalities of Puerto Rico (Adjuntas, Coamo, Corozal, Juana Diaz, Lajas, Mayaguez, Morovis, Orocovis, Santa Isabel, Villalba). One hundred percent of the samples were positive for at least one of the tested viruses. Serologic tests showed more than 90 percent of the samples to be positive for potyvirus, with 45% positive for PRSV and 40% positive for ZYMV. Ten percent of the samples were from an unknown potyvirus. WMV, SqMV and CMV were reported in less than 2 percent of the samples. Occurrence of superinfection with 2 or more viruses was common. Mechanical transmission to *Cucurbita moschata* 'Waltham' was conducted and expression of symptoms was observed 2 weeks after inoculation. RNA was extracted from field samples and inoculated plants. RT-PCR was conducted with potyvirus degenerate primers that amplify a coat protein gene fragment and the amplicons were sequenced to identify more specifically the potyviruses infecting the cucurbits. The information has been used to select virus strains to challenge resistant pumpkin lines in a current breeding program and to optimize the selection of mild strains for cross protection.

Occurrence of a whitefly transmitted *Carlavirus* in soybean in Puerto Rico

J. V. RODRIGUES (1), D. Viteri (1), C. Estévez de Jensen (1), E. W. Kitajima (2)
(1) University of Puerto Rico, Department of Crop Protection, Mayaguez, PR; (2) University of Sao Paulo, Piracicaba, SP, Brazil

Phytopathology 98:S134

Soybean is cultivated in Puerto Rico in summer nurseries to advance breeding programs by several seed companies. In Puerto Rico symptoms of leaf vein and stem necrosis and plant stunting were observed in soybean experimental field plots in municipalities of Juana Diaz, Santa Isabel and Isabela. Observations in a winter nursery in Santa Isabel showed 100 percent incidence in some soybean lines, indicating the potential of damage of this disease in soybeans. Samples were collected and transmission assays were conducted at Rio Piedras Experimental Station. Mechanical inoculation, stem grafting and whitefly (*Bemisia tabaci*) transmission resulted in development of the same symptoms as those observed in the field. All assays were conducted using the soybean line 2053A. Leaf tissues were pre-fixed in Karnovsky's fixative and processed for transmission electron microscopy. Ultrathin sections of diseased tissues showed in the cytoplasm the occurrence of brush-like inclusions, which have been reported before in association with *Carlavirus* infections. RNA was extracted from symptomatic and healthy plants. RT-PCR using specific primers available for *Carlavirus* amplified a fragment of the expected size only from symptomatic plants. Full characterization of the virus, host range and soybean resistance are needed.

Comparison of field, tuber and detached leaf evaluations of potato germplasm for late blight resistance

J. J. RODRIGUEZ (1), A. L. Thompson (2), V. Rivera-Varas (1), G. A. Secor (1)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA; (2) Department of Plant Science, North Dakota State University, Fargo, ND, USA

Phytopathology 98:S134

Three *Phytophthora infestans* (Pi) resistance assessment methods were tested in sixty potato breeding selections from selected parents with reported resistance to Pi to correlate foliar and tuber resistance. Assessment methods were: (a) tuber inoculation, (b) foliar field, and (c), foliar greenhouse by detached leaf assay (DLA). DLA and tuber assays were conducted in replicated trials in two years using an inoculum suspension of 2×10^4 zoospores ml^{-1}. Tubers were inoculated with a 10 µl of Pi inoculum in three eyes on the bud end, incubated for 14 days and evaluated for lesion severity. For the DLA, leaves were inoculated with 30 µl of inoculum and after five days of incubation, rated for disease using a zero to four scale. In the field, plants were inoculated and evaluated weekly for disease severity and an AUDPC calculated for each selection. A significant difference in genotype reaction to the disease was observed by the three methods. Selections ND8527B-94, ND028945B-4, ND028801CB-1, ND8277B-5 and ND6961B-21PY showed high levels of resistance to Pi by all three methods. One year of foliar field data did not correlate with tuber ($r = 0.27$ $P < 0.033$) or DLA ($r = 0.39$ $P < 0.016$ for 2007 and $r = 0.30$ $P < 0.017$ for 2008), however, DLA was able to detect 84.7% of the selections that showed high to moderate resistance in the field, and may be useful as a screening method for predicting Pi resistance in families from directed crosses for late blight resistance.

Effectiveness of fungicide seed treatments against seed-borne *Fusarium verticillioides* in maize (*Zea mays* L.)

C. RODRIGUEZ-BRLJEVICH (1), A. E. Robertson (1), D. J. Nordman (1)
(1) Iowa State University, Ames, IA 50011, USA

Phytopathology 98:S134

The effectiveness of various fungicide active ingredients against *F. verticillioides* seed-borne inoculum was assessed under controlled conditions (13°C and 12 h light/dark). Maize seed, inoculated with *F. verticillioides* TXI-79, an isolate labeled with genes for green fluorescent protein and Hygromycin B resistance, was treated with either fludioxonil (2.5 g ai 100 kg/seed), azoxystrobin (1 g ai 100 kg/seed), captan (71.1 g ai 100 kg/seed), trifloxystrobin (10 g ai 100 kg/seed), A10466C (20 g ai 100 kg/ha), ipconazole (2.5 g ai 100 kg/seed) or triticonazole (10 g ai 100 kg/seed). The incidence of TXI-79 was assessed in seed at 7 days after planting (dap) and in radicle and hypocotyl tissues at 7, 14 and 21 dap by plating seed, radicle and hypocotyl dissections onto Nash Snyder medium amended with Hygromycin B. No radicle or hypocotyl rot symptoms were observed in seedlings. Treatments containing A10466C consistently eradicated seed-borne TXI-79 from seed, radicle and mesocotyl tissues. Ipconazole and triticonazole reduced the incidence of TXI-79 in all seedling tissues examined by up to 90%. Overall, strobilurins active ingredients were less effective against seed-borne TXI-79 than triazoles.

Fungicide seed treatments reduce infection of maize by soil-borne *Fusarium* species and thereby contribute to improved photosynthesis

C. RODRIGUEZ-BRLJEVICH (1), A. E. Robertson (1), C. Kanobe (1), D. J. Nordman (1), J. F. Shanahan (2)
(1) Iowa State University, Ames, IA 50011, USA; (2) University of Nebraska-Lincoln

Phytopathology 98:S134

Maize seed treated with either Cruiser Extreme® 250 [fludioxonil (2.5 g ai/100 kg seed) + azoxystrobin (1 g ai/100 kg seed) + thiamethoxam (62.5 g ai/100 kg seed) + mefenoxam (2 g ai/100 kg seed)] or a premix of Vortex®-Trilex® FL-Allegiance® FL [ipconazole (2.5 g ai/100 kg seed)+ trifloxystrobin (10 g ai/100 kg seed) + metalaxyl (28 g ai/100 kg seed)], was planted at Southeast Research Farm, Crawfordsville, Iowa and Northeast Research Farm, Nashua, Iowa. Fungicide seed treatments effects on early season growth and physiology were evaluated by assessing incidence of *Fusarium* species colonization, chlorophyll fluorescence (CF) (estimate of photosynthetic performance) 14, 28 and 52 days after emergence (dae). Plant height was assessed at 28 and 52 dae. The most prevalent species isolated at both locations were *F. graminearum* and *F. subglutinans*, followed by *F. verticillioides* at Nashua and *F. proliferatum* at Crawfordsville. Photosynthetic performance, as measured by CF, decreased with increased incidence of *Fusarium* species at 28 and 52 dae (Nashua, $r^2 = –97$, $r^2 = –0.74$; Crawfordsville, $r^2 = –0.71$, $r^2 = –0.81$, respectively). Cruiser Extreme® 250 seed treated plants had the highest ($P < 0.05$) CF values and lowest ($P < 0.05$) incidence of *Fusarium* species colonization at both locations at 52 dae. CF measurements positively correlated with plant height measurements (Nashua $r^2 = 0.64$; Crawfordsville $r^2 = 0.57$) at 52 dae.

Study of the genetic diversity of *Phytophthora infestans* isolates from the Northern Andean region using seven genic regions

A. ROJAS (3), R. Sierra (3), A. Gonzalez (3), A. Vargas (3), M. Cárdenas (3), A. Grajales (3), C. Salazar (3), M. Marín (1), G. Fermin Munoz (4), L. E. Lagos (2), A. Bernal (3), S. Restrepo (3)
(1) Universidad Nacional, Medellin, Antioquia, Colombia; (2) Universidad de Nariño, Pasto, Nariño, Colombia; (3) Universidad de los Andes, Bogotá D.C., Colombia; (4) Universidad de los Andes, La Hechicera, Merida, Venezuela

Phytopathology 98:S134

Phytophthora infestans, the causal agent of potato late blight, has been extensively studied in the past few years in terms of the molecular strategies it uses to cause disease in plants. *P. infestans* is one of the most important pathogens in Colombia and the area used for cultivation of potato has increased considerably. Additionally, we have recently reported the disease in cape gooseberry, a crop usually cultivated next to potato fields. This underscores the importance of characterizing the population structure of this pathogen. Using a phylogenetic approach, we analyzed the diversity present

on Colombia, Ecuador and Venezuela, to get an insight on the genetic diversity of this pathogen. Sequences were obtained for 96 isolates for seven genic regions with both nuclear (pex147, SCR74, Ras - IntronRas, B tubulin and Internal transcribed spacer) and mitochondrial origin (cox I). Phylogeny and genealogy analyses were used to describe the population structure. We determined correlations between molecular differences and geographical localization and host origin. We observed a reduced number of polymorphisms in most of the genes, although virulence genes, which are supposed to be under selection pressure, showed a higher number of polymorphic sites. This work greatly contributes to determine the origin of the diversity *P. infestans* by establishing migration analysis.

Geographical diversity of the grapevine pathogen *Eutypa lata* in North American vineyards

P. ROLSHAUSEN (4), K. Baumgartner (5), S. Bergemann (1), P. Fujiyoshi (5), D. Gubler (3), W. Wilcox (2)
(1) Department of Biology, Middle Tennessee State University, Murfreesboro, TN, USA; (2) Department of Plant Pathology, Cornell University, Geneva, NY, USA; (3) Department of Plant Pathology, University of California, Davis, USA; (4) Department of Plant Science, University of Connecticut, Storrs, USA; (5) USDA-ARS, Davis, CA, USA
Phytopathology 98:S135

Eutypa lata ascospores strictly contribute to the spread of Eutypa dieback of grapevine. Conidia of the fungus are produced in nature, but are not infectious. Ascospores are released from perithecia following rain and are wind-dispersed. They infect grapevine vascular tissue by colonizing susceptible wounds (pruning wounds, freeze-damaged tissue). Past research on the spread of Eutypa dieback supports both local and distant origins of ascospore inoculum in individual vineyards. However, the epidemiology of this disease was mainly studied in Mediterranean regions. The timing of spore production and infection is not known in cold climates, where freezing winter temperatures may restrict dormant season spore dispersal, temporally and spatially. Our objective was to examine the diversity of *E. lata* populations from North American vineyards in regions representing both Mediterranean and cold climates. To determine the frequency of gene flow within and between vineyards, we isolated and characterized nine *E. lata*-specific microsatellite markers. *Eutypa lata* was collected from diseased vineyards located in California, Connecticut, New York and Rhode Island. Cultures were initially identified based on culture morphology and DNA sequencing of the rDNA internal transcribed spacer region (ITS). Despite similar culture morphology and minimal sequence differences among a total of 95 isolates, not all microsatellite markers amplified DNA from all isolates. Our findings of unique alleles among East and West coast populations of *E. lata*, in addition to a low frequency of gene flow suggest that East and West coast populations are somewhat isolated from each other.

Integrated Pest Management in the Cuban tobacco crops

C. R. ROMERO (3), E. Garcia (2), M. Domínguez (3), L. E. Blanco (3), E. Capote (4), C. Mandariaga (1)
(1) Plant Health National Center; (2) Sugar Cane Research Institute; (3) Tobacco Research Institute, Plant Pathology Department, San Antonio de lo Baños, Habana Cuba; (4) Trading Tobacco Company
Phytopathology 98:S135

The Tobacco Research Institute and the Plant Health National Centre has been working for many years to create an efficient Integrated Pest Management (IPM) program to the tobacco farmers in Cuba. This program consists on the five basic components that including pest identification. The identification is carried out by labs that belong to the surveillances plant health system in which there is a good experience applying traditional and advanced techniques for pathogens detections. Another IPM component is a monitoring or scouting of the phytopathogens that is based on the study of pathogen population dynamics (it presence and density on the fields). The monitoring includes the risks analysis of the potential pathogens that could attack in the future the tobacco crops. These bellow considerations help us to estimate the economic losses estimation related with the plant disease incidence in the tobacco fields. This threshold determination is coordinated with the most convenient control methods to keep the pests level as low as possible. For that reason we have also applied a physical control like crop rotation and biological control using the natural enemies, to regulate the pest populations. We also applied the chemical control measures which it is important to understand the life cycle of the pathogen so that the pesticide can be used when the pest is at its most vulnerable. Within the IPM of the obtaining of resistant cultivars or varieties is very important subject. We have made a plant breeding programs that started with a creation of new genotypes (genetic combinations) through hybridization and continues with an evaluation and selection of some superior genetics combinations. We have developed new tobacco varieties that has been tested in various locations for several years and evaluated for characteristics related with to productivity, processing quality, storability and marketability. In our case we don't use any genetic DNA tobacco plant manipulations techniques.

Defining the role of RTX toxins in virulence of *Pantoea stewartii* subsp. *stewartii*, the causal agent of Stewart's wilt of corn

M. ROPER (1), S. Von Bodman (1)
(1) Department of Plant Science, University of Connecticut, Storrs, CT 06269
Phytopathology 98:S135

Pantoea stewartii subsp. *stewartii i*s a serious pathogen affecting sweet corn and maize. This bacterium colonizes the xylem tissue and produces large amounts of stewartan EPS, the major virulence factor contributing to plant wilt. The *P. stewartii* genome possesses two genes with homology to large repetitive RTX toxins. RTX toxins comprise a large family of lytic proteins that are widely distributed among gram-negative bacteria. RTX toxins are well described for mammalian pathogens, but little is known about their role in plant pathogenesis. During the initial stages of infection, *P. stewartii* creates water-soaked lesions. This is due, in part, to WtsE, a type III effector secreted by the Hrp type III secretion system. We hypothesize that RTX toxins also play a role in the water-soaking phase of infection because of their lytic properties. Moreover, the promoters for *rtx1* and *rtx2* contain potential binding sites for ArcA and OxyR, transcription factors that are activated under microaerobic conditions and oxidative stress, respectively. These are conditions that *P. stewartii* likely encounters during the initial phase of plant infection. We envision that RTX toxins are important virulence factors when *P. stewartii* initially enters the plant tissue. In this study we constructed deletion mutants in the two *P. stewartii rtx* genes by marker exchange mutagenesis and tested them for virulence and water-soaking *in planta*. These data indicate that one of the RTX toxins does contribute to the characteristic water-soaked symptom and is an important virulence factor for *P. stewartii.* Therefore, RTX toxins are one strategy that *P. stewartii* employs during the initial infection of sweet corn.

Big vein disease (BVD) of lettuce: Studies to measure its incidence, variation for symptom expression and role of the antioxidant system in the course of the disease

I. ROSALES (1), C. Araya (1), L. Roman (1), E. Peña (1), R. Mora (1), E. Salazar (1), A. Aljaro (1)
(1) Instituto de Investigaciones Agropecuarias (Inia), Plant Breeding and Biotechnology Department, Santiago, Rm, Chile
Phytopathology 98:S135

Big vein is an economically damaging disease complex of lettuce (*Lactuca sativa* L.) that occurs in all lettuce-producing areas. This disease involves two different viruses: Mirafiori lettuce big-vein virus (MLBVV) and Lettuce big-vein associated virus (LBVaV), both transmitted by the soil-borne fungus *Olpidium brassicae*. The disease is difficult to control since the resting spores of the fungus can persist for over 20 years in soil and because there are no major resistance genes available in commercial varieties of lettuce. In Chile, typical symptoms of the disease were first described in the year 2003. We have conducted a survey to measure the occurrence and distribution of this disease in approximately 1000 samples collected during years 2006 and 2007 in the major lettuce-growing areas of central Chile. The results showed that BVD is present in all regions surveyed with an overall incidence of 50%. Variety trials were conducted two consecutives years to evaluate the agronomic performance and variation for big vein symptom expression in 127 lettuce varieties belonging to four different types (cos, butterhead, iceberg and leaf types). All varieties tested were susceptible to virus infection, but there was a high symptom variation among lettuce groups and varieties, being the cos and iceberg types the most affected. Additionally, we studied the changes in the activities of antioxidant enzymes (CAT, SOD, APX) and some indicators of oxidative damage in response to virus infection in three lettuce varieties: Sharp Shooter, Winterhaven and Pavane. We did not find major influence of the virus infection on enzymes activities or lipid peroxidation in all varieties studied. However, the photosynthetic pigment contents (chlorophyll a and b) were significantly decreased in the susceptible varieties Sharp Shooter and Winterhaven but not in the tolerant variety Pavane. This research was supported by projects: FIA-PI-C-2005-1-A-051 and FONDECYT INICIACIÓN N°11060173.

Begomovirus infecting tomato crops in the north of Chile

I. ROSALES (3), G. Sepulveda-Chavera (2), M. Rojas-Jara (2), C. Sandoval (1)
(1) Departamento de Producción Agrícola, Facultad de Ciencias Agrarias, Universidad de Talca, Talca, Chile; (2) Departamento de Recursos Ambientales, Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, Chile; (3) Instituto de Investigaciones Agropecuarias (Inia), Plant Breeding and Biotechnology Department, Santiago, Rm, Chile
Phytopathology 98:S135

Begomoviruses affect many important food and industrial crops in LatinAmerica, but until now, they had not being described in the Chilean

territory. During 2007, symptoms of yellowing, chlorotic curled leaves and stunted plants associated with high levels of whitefly populations were observed in tomato fields in the Region of Arica and Parinacota, in the north of Chile. Symptomatic plants were collected in open fields and greenhouse tomatoes from different locations in the Azapa Valley. Tissue from seventy affected plants was used for total DNA extraction and polymerase chain reaction (PCR) amplification using 3 set of primers: a) the degenerate primers prV324/ prC889 for universal detection of geminivirus described by *Wyatt and Brown* (1996) which direct the amplification of a 576 - 579 bp from the viral coat protein, b) universal degenerated primer set PAL1v1978/PAR1c496 that allows the amplification approximately 1,1 kb of mono and bipartites begomoviruses, described by *Rojas et al*. (1993), and c) primers set PCRc1/PBL1v2040 described by *Rojas et al*. (1993) which amplifies a fragment of approximately 500 bp from the DNA-B segment. In all cases, we have obtained the expected PCR fragment after the amplification. PCR products were cloned and sequenced. Until this moment, we have obtained the sequence of over 600 bp from the 1,1 kb amplicon generated with primers PAL1v1978/PAR1c496. This viral DNA was compared with DNA sequences available at NCBI database using Blast option, and the sequence similarity search matched the bipartite begomovirus "Tomato yellow vein streak virus" with nucleotide sequence identities of 97%. We will continue obtaining the sequences from other amplicons and samples to discard the presence of other begomoviruses in the region. Support for this research comes from project FIA-PI-C-2005-1-A-03, Natural Resources Department from Universidad Tarapacá, and Instituto de Investigaciones Agropecuarias (INIA).

Molecular approaches for taxa discovery in plant-associated soil microbial communities

N. ROSENZWEIG (1), J. Kang (1), L. L. Kinkel (1), J. M. Bradeen (1)
(1) Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S136

Plants influence rhizosphere microbial community composition, but there is little systematic information on the variation in microbial communities associated with different plant hosts. This work explores the challenges and limitations of current molecular techniques in resolving microbial community structure. Soil cores (n = 10 for each plant species) were collected from the rhizosphere of vigorous and well-established *Andropogon gerardii* (Ag) and *Lespedeza capitata* (Lc) plants at the Cedar Creek Ecosystem Science Reserve in east-central Minnesota. Ribosomal 16S V3 fragments from rhizosphere bacterial communities were studied using denaturing gradient gel electrophoresis (DGGE) and targeted sequencing. DGGE patterns were unable to distinguish microbial communities associated with environmental samples from each plant species. To evaluate the sensitivity of DGGE, 'communities' of varying complexity were simulated by mixing different bacterial clones. Only slight DGGE banding differences were observed between phylogenetically distinct taxa. Targeted sequencing of the 16S V3 region revealed differences in microbial communities associated with Ag and Lc, though the data are limited by the small sample sizes possible with traditional cloning approaches. These results indicate significant limitations in the use of DGGE for resolution of complex microbial communities. As an alternative, we are developing a novel resource of diversity-enriched clone libraries and pursuing large-scale sequencing of 16S V3 region fragments. Our combined approaches will yield a resource-efficient means of characterizing complex environmental samples and discovering rare and under-represented taxa.

Quantitative analysis of tomato spotted wilt virus (TSWV) titer in *Frankliniella occidentalis* and its association with frequency of transmission

D. ROTENBERG (3), N. K. Krishna Kumar (4), A. E. Whitfield (3), M. Montero-Astúa (3), D. K. Willis (5), T. L. German (2), D. E. Ullman (1)
(1) Department of Entomology, University of California, Davis, CA; (2) Department of Entomology, University of Wisconsin, Madison, WI; (3) Department of Plant Pathology, Kansas State University, Manhattan, KS; (4) Indian Institute of Horticultural Research, Bangalore, India; (5) United States Department of Agriculture, Vegetable Crops Research Unit, Madison, WI
Phytopathology 98:S136

Tomato spotted wilt virus (TSWV) is transmitted in a persistent propagative manner by *Frankliniella occidentalis*, the Western flower thrips (WFT). While it is well established that vector competence depends on TSWV acquisition by young larvae and virus replication within the insect, the biological factors associated with frequency of transmission have not been well characterized. We hypothesized that frequency of transmission by adult thrips is determined, in part, by the amount of virus harbored (titer) by the insect. Transmission time-course experiments were conducted using a leaf disk assay to determine the efficiency and frequency of TSWV transmission following 2-day inoculation access periods (IAPs). Virus titer in individual adult thrips was determined by real-time reverse transcriptase (RT) – PCR at the end of the experiments. Over the adult life-span, the efficiency of transmission was highest within 7 days post adult-eclosion, and decreased over time. On average, 51% of the population transmitted the virus during the first IAP, 2-3 days post adult-eclosion. Examination of virus titer in individual insects at the end of the third IAP (7 days post adult-eclosion) revealed significant and consistent positive correlations between frequency of transmission and virus titer. Our data support the hypothesis that a viruliferous thrips is more likely to transmit multiple times if it harbors a high titer of virus.

A diffusible signal factor modulates albicidin biosynthesis by *Xanthomonas albilineans*

P. C. ROTT (1), S. L. Reddy (3), G. C. Marlow (3), M. Royer (2), D. W. Gabriel (3)
(1) CIRAD UMR BGPI, Montpellier, F-34398 France and University of Florida, Plant Pathology Department, Gainesville, FL 32611; (2) CIRAD UMR BGPI, Montpellier, F-34398 France; (3) University of Florida, Plant Pathology Department, Gainesville, FL 32611
Phytopathology 98:S136

Xanthomonas albilineans produces albicidin, a unique and specific toxin that causes foliar symptoms of sugarcane leaf scald disease. In the related species *Xanthomonas campestris* pv. *campestris*, a cluster of genes called *rpf* (for ***r***egulation of ***p***athogenicity ***f***actors) is involved in control of various cellular processes. Marker exchange mutants of the *rpfF* gene in strain XaFL07-1 of *X. albilineans* from Florida were generated, and the mutants were verified by PCR analysis and by use of an *X. campestris* diffusible signal factor (DSF) reporter strain. Both albicidin and protease production by these mutants appeared significantly reduced compared to XaFL07-1, suggesting that DSF is involved in regulation of albicidin biosynthesis by *X. albilineans*. However, sugarcane cultivar CP80-1743, moderately susceptible to leaf scald, exhibited pencil line symptoms indicative of albicidin production on emerging leaves after inoculation of stalks by the decapitation method. Preliminary experiments indicated that at least some *rpfF* mutants colonized sugarcane stalks less efficiently, both spatially and in intensity, than wild type *X. albilineans*. Additional inoculation experiments are in progress to assess disease severity caused by *rpfF* mutants, and to study the role of DSF production in sugarcane stalk colonization by *X. albilineans*.

Impact of episodic root stress on the susceptibility of *Rhododendron* sp. and *Viburnum tinus* to *Phytophthora ramorum*

T. V. ROUBTSOVA (1), R. M. Bostock (1)
(1) University of California, Davis, Dept. Plant Pathology, Davis, CA, USA
Phytopathology 98:S136

Phytophthora ramorum attacks members of the Fagaceae, causing foliar blight and dieback on many forest and nursery species. To examine root infection by *P. ramorum* and the potential role of mild abiotic stress in disease predisposition, experimental systems were established with *Rhododendron sp*. and *Viburnum tinus.* Experiments were conducted in two formats: modified hydroponic culture and standard potting soil. Roots of plants were exposed to NaCl stress prior to inoculation under four treatment regimes: 1) salt-stressed, non-inoculated; 2) non-stressed, non-inoculated; 3) salt-stressed, inoculated; and 4) salt-stressed, non-inoculated. Plants in hydroponic culture were exposed to 0.2 M NaCl/0.02 M CaCl2/0.5x Hoaglands for 12 hours and then returned to 0.5x Hoaglands. Potted plants were treated with a soil drench of 0.2 M NaCl/0.02 M CaCl2 for 12 hours, and then flushed with water to remove the salt. Roots were then inoculated with zoospores of *P. ramorum*. In hydroponic plant cultures, the two *P. ramorum* isolates tested were similar in pathogenicity on *Rhododendron* and *Viburnum* plants, with root and stem lesions developing within one week after inoculation (10^4 zoospores/ml) in salt-stressed roots. Non-stressed, inoculated plants became symptomatic after two weeks. Microscopic examination of roots from both species revealed that their tips were covered with sporangia of *P. ramorum*. On potted *Rhododendron* plants, disease developed in salt-stressed roots, with death of the plant occurring within four weeks after inoculation. Non-stressed plants survived for 6–8 weeks following inoculation. The implications of episodic stress in root infection by *P. ramorum* and disease development in nursery ornamentals will be presented.

Stem rust resistance in *Triticum monococcum* germplasm

M. N. ROUSE (2), Y. Jin (1)
(1) USDA-ARS Cereal Disease Laboratory, St. Paul, MN, USA; (2) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S136

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has been effectively controlled through the use of genetic resistance. The recently identified race TTKSK (Ug99) possesses virulence to many resistance genes that have been used in wheat breeding worldwide. One strategy to aid breeders in developing resistant varieties is to provide resistance genes transferred from wild relatives to wheat. Stem rust resistance genes *Sr22* and *Sr35*, derived from *Triticum monococcum* are effective against race TTKSK. In order to identify additional genes from this wild relative of wheat, we screened 1062 accessions deposited in the National Small Grains Collection

against TTKSK and two additional races with broad virulence. We identified 625 accessions (58.85%) with resistance to TTKSK with infection types ranging from 0 to 2+. Among these resistant accessions, 90 accessions (8.47% of the total) were also resistant to TTTT and TRTT. Results from the preliminary screening suggested that novel resistance genes to race TTKSK are likely to be present in *T. monococcum*. These resistant accessions are being characterized further by testing with additional stem rust races. Crosses among selected resistant *T. monococcum* accessions have been initiated to determine the number and allelic relationships of stem rust resistance genes.

Diversity of a disease resistance gene homolog in natural populations of *Andropogon gerardii* (Poaceae) is correlated with precipitation

M. N. ROUSE (1), A. A. Saleh (1), K. H. Keeler (3), S. E. Travers (2), S. H. Hulbert (4), K. A. Garrett (1)
(1) Kansas State University, Manhattan, KS, USA; (2) North Dakota State University, Fargo, ND, USA; (3) University of Nebraska, Lincoln, NE, USA; (4) Washington State University, Pullman, WA, USA
Phytopathology 98:S137

Ecological clines often result in gradients of disease pressure in natural plant communities, imposing a gradient of selection on disease resistance genes. We describe the diversity of a resistance gene homolog in natural populations of the dominant tallgrass prairie grass, *Andropogon gerardii*, across a precipitation gradient ranging from 47.63 cm/year in western Kansas to 104.7 cm/year in central Missouri. Since moisture facilitates infection by foliar bacterial pathogens, plants along this precipitation gradient will tend to experience heavier bacterial disease pressure to the east. In maize, the gene *Rxo1* confers resistance to the pathogenic bacterium *Burkholderia andropogonis*. *Rxo1* homologs have been identified in *A. gerardii* and *B. andropogonis* is known to infect natural populations of *A. gerardii*. The spatial genetic structure of *A. gerardii* was assessed from central Missouri to western Kansas by genotyping with AFLP markers. Samples were also genotyped for *Rxo1* homologs by amplifying an 810 base pair region of the leucine-rich repeat and digesting with restriction enzymes. We compared *Rxo1* homolog diversity to AFLP diversity across different spatial scales. Genetic dissimilarity based on AFLP markers was lower than would have occurred by chance at distances up to 30 m, and different prairies were more dissimilar than would have occurred by chance, but there was not a longitudinal trend in within-prairie dissimilarity as measured by AFLP markers. Dissimilarity of the *Rxo1* homologs was higher in the east suggesting the presence of diversifying selection in the more disease-conducive eastern environments.

Efficacy of biological and other novel seed treatments suitable for use in organic peanut production systems

S. J. RUARK (1), B. B. Shew (1)
(1) North Carolina State University, Raleigh, NC, USA
Phytopathology 98:S137

Planting untreated peanut seed often results in stand losses >50% and poor stands are a constraint on organic production. Biological, other novel seed treatments, and soil amendments were tested for efficacy against pre- and post-emergence damping-off. Seed of the lines Perry, GP-NC 343, and N03081T were planted in natural soil in all trials. A total of 22 treatments were tested in three greenhouse trials. In two tests, no treatment increased emergence or reduced damping-off compared to the untreated control. In the third test, *Bacillus subtilis* (Kodiak) and copper hydrate (Champion) performed as well as a standard seed treatment fungicide. A field microplot study evaluated five treatments on the three peanut lines following wheat, oat, or triticale cover crops, soil amendment with *Muscodor albus*, or a no cover control. The incidence of damping-off depended on peanut line × treatment interactions. N03081T had high germination regardless of treatment. Emergence of GP-NC 343 and Perry was lowest with *Trichoderma harzianum* (T22) but no treatment was better than the untreated control. Cover crops did not affect emergence, but *M. albus* treatment suppressed emergence. In a field study at Lewiston NC, the three peanut lines were treated with *M. albus*, Kodiak, T22, or were untreated. Stand varied among lines, but none of the treatments improved stands compared to the untreated check. The predominant pathogen was *Aspergillus niger*. A greenhouse test was conducted with natural soil or soil infested with field isolates of *A. niger*. Emergence and survival was much lower in infested vs uninfested soil. Kodiak and Champion reduced damping-off compared to untreated seed.

Viral host factor MPB2C plays a role in cortical microtubular assemblies, stomata patterning and tobamovirus infectivity

P. Ruggenthaler (1), D. Fichtenbauer (1), E. WAIGMANN (1)
(1) Max F. Perutz Laboratories, Medical University of Vienna, Vienna, Austria
Phytopathology 98:S137

AtMPB2C is the *Arabidopsis thaliana* homologue of MPB2C, a microtubule associated host factor of tobacco mosaic virus movement protein that has been previously identified in *Nicotiana tabacum*. To analyze the endogenous function of AtMPB2C and its role in viral infections, transgenic *Arabidopsis* plant lines stably overexpressing GFP-AtMPB2C were established. The GFP-AtMPB2C fusion protein was detectable in various cell types and organs and localized at microtubules in a punctuate pattern or in filaments. In rapidly elongated cell types such as vein cells and root cells, overexpression of GFP-AtMPB2C caused highly unordered assemblies of cortical microtubules, a disturbed, snake-like microtubular shape and star-like crossing points of microtubules. Phenotypically, GFP-AtMPB2C transgenic plants showed retarded growth but were viable and fertile. Seedlings of GFP-AtMPB2C transgenic plants were characterized by clockwise twisted leaves and clustered stomata. GFP-AtMPB2C overexpressing plants showed increased resistance against oilseed rape mosaic virus but not against cucumber mosaic virus when compared to *Arabidopsis* wild type plants. These results suggest that AtMPB2C is involved in the alignment of cortical microtubules, the patterning of stomata and in restricting tobamoviral infections. This work was funded by WWTF (project LS123), FWF (Sfb17 project part 08) and ÖAD (project 18/2006) to E.W.

Effect of apple scab fungicide programs on colonization and survival of *Botryosphaeria* spp. in mummified apple fruitlets in NY

N. L. RUSSO (1), D. A. Rosenberger (2), K. D. Cox (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY 14456; (2) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Highland, NY 12528
Phytopathology 98:S137

Botryosphaeria obtusa and *B. dothidea* are the causal agents of black rot and white rot of apple, respectively. Mummified fruitlets retained in the canopy following thinning are believed to serve as a source of primary inoculum. The objective of the study was to evaluate the effect of 13 apple scab fungicide programs on the retention and *Botryosphaeria* colonization of mummified fruitlets. In 2006 and 2007, mummified fruitlets were collected from orchard sites in western (Geneva) and eastern NY (Highland). All mummified fruitlets were collected from four singletree replicates and placed in one of four size categories. Isolations were made from a subset of mummified fruitlets from each size category within treatment replicates to determine the level of *Botryosphaeria* infection. Fungicide programs did not significantly affect mummy retention in 2006 or 2007, at either location. Mummy size was not a significant factor in colonization, indicating small florets as well as large fruitlets serve as sources of inoculum. In 2007 at the Geneva location a Captan+Dithane program was the most effective at reducing *Botryosphaeria* colonization. Dithane, Nova, and Nova+Dithane programs had reduced *Botryosphaeria* colonization, while Sovran+Flint and Inspire programs were the least effective in reducing colonization. Reduction in primary inoculum through the use of an appropriate fungicide could reduce late season losses associated with *Botryosphaeria* spp. and future epidemics.

Development of black spot symptoms in fruits of Nova tangerine

M. A. RYBAK (2), B. I. Canteros (2), M. G. Cabrera (1)
(1) Facultad de Ciencias Agrarias de la Universidad Nacional del Nordeste, Corrientes, Argentina; (2) INTA-BID 1728 OC/AR PICTO 12956 EEA INTA, Bella Vista, Corrientes, Argentina
Phytopathology 98:S137

Development of symptoms of citrus black spot, caused by Guignardia citricarpa (Kiely), were observed in a plot of Nova tangerine (Citrus reticulata Blanco) of ten-years-old-trees starting in March 2007 and until harvest. The grove is located at INTA Bella Vista, Corrientes, Argentina, and did not receive any spray for disease control. The fast and early development of symptoms was never observed before in this growing region, especially in young trees. All fruits were rated for disease severity at three dates: March 14, April 13, and May 15. A commonly used three degree scale was used: healthy (1), low infection (2), and high(2) severity. All fruits of each plant were observed. At the earliest date 11% of all plants showed symptoms. Diseased fruits were 36% in degree1 and 64% degree 2. In April, 88% of plants were affected, and the diseased fruits were 76.5% degree 1, and 23.5% degree 2. When all fruits were harvested at fruit maturity 100% of trees have diseased fruits: 25% without symptoms (degree 0), 40% degree1, and 35% degree 2. Nova tangerine was widely planted in the last years and most groves are very young and no-symptoms of black spots were observed previously in trees this age. The fast development of symptoms in an early maturing variety suggest that programs of sprays should be determined to control black spot in Nova tangerine.

A novel marafivirus from Rubus spp.

S. SABANADZOVIC (1), N. Abou Ghanem-Sabanadzovic (1)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, USA
Phytopathology 98:S137

A survey was conducted to identify viruses present in the native Rubus spp. population in the Great Smoky Mountain National Park. Four specimens of

symptomatic smooth blackberry (syn. Canadian blackberry, Rubus canadensis L.) displaying chlorosis, vein yellowing and leaf deformation reacted positively in RT-PCR when tested with universal primers for viruses belonging to the family Tymoviridae. Amplicons from all four samples were cloned and sequenced. Analyses showed high sequence conservation (96–99% identity) among clones from different specimens, indicating infection by the same virus in all tested samples. Computer analysis of the viral genome revealed the presence of a single, long open reading frame resembling the marafivirus genome. BLAST search showed that the virus present in tested samples is putatively a new member of the genus Marafivirus that shares ca 55–60% common aminoacids with the polyproteins encoded by genomes of Citrus sudden death-associated virus (CSDaV), Maize rayado fino virus (MRFV) and Oat blue dwarf virus (OBDV). Virus-specific primers were designed in order to study the etiological role of this virus in the disease and its incidence in wild and cultivated Rubus spp.

An undescribed dsRNA virus from Rhododendron

S. SABANADZOVIC (1), N. Abou Ghanem-Sabanadzovic (1), D. L. Gutierrez (2), R. A. Valverde (2)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, USA; (2) Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, Baton Rouge, LA 70803, USA
Phytopathology 98:S138

Foliar samples of Rhododendron maximum L. (Great Rhododendron, Great Laurel, Big Rhododendron) were collected at different locations in Great Smoky Mountain National Park and assayed for the presence of RNA viruses by dsRNA analysis. A dsRNA molecule of an estimated size of 3.5 kbp was detected in some of the samples, most of them without disease symptoms. Purified dsRNAs were used as a template for cloning and sequencing. Sequence data indicated that the dsRNA represents the genome of a novel plant virus for which the name Rhododendron virus A (RhVA) is proposed. The genome consisted of ca. 3,430 bp and contained two partially overlapping open reading frames (ORFs). Both ORFs were phylogenetically related with orthologous genes present in the recently described Tomato yellow stunt-associated virus and Blueberry fruit drop virus. The three viruses clustered together forming an independent clade among dsRNA viruses implying that they may belong to a yet-to-be-established taxon of phytoviruses. PCR tests using ITS-specific primers did not reveal the presence of an endophytic fungus. This confirms that rhododendron is the host for this new dsRNA virus.

Detection and identification of an umbravirus from Ageratina altissima

S. SABANADZOVIC (1), N. Abou Ghanem-Sabanadzovic (1)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, USA
Phytopathology 98:S138

Random-primed cloning of reverse transcribed dsRNAs extracted from white snakeroot (Ageratina altissima L. King &H. Rob.) specimens showing chlorotic spots and mosaic symptoms yielded several clones of phytoviral origin. Comparison of initial data with the sequences available in GenBank showed that the virus had a close relationship with members of the genus Umbravirus. The complete genome of this virus showed the presence of four open reading frames (ORFs), the genomic organization resembling that of umbraviruses. ORFs 1 and 2 are likely translated via a -1 frameshift mechanism as a single polypeptide involved in viral replication, whereas 3′-proximal overlapping ORFs 3 and 4 enable cell-to-cell and long distance movements within the plant. Viral polymerase was closest to Groundnut rosette virus (65% identical residues), while proteins encoded by ORF3 and 4 showed 30–40% identities with orthologous products of Pea enation mosaic virus-2 (PEMV-2) and Tobacco mottle virus (TMoV) respectively. According to the overall molecular data, the virus from Ageratina is likely an undescribed member of the genus Umbravirus for which the name Ageratina chlorotic spot virus (AgCSV) is proposed. As umbraviruses rely on a specific helper virus for transmission by aphids, the research on identification of a virus associated with AgCSV is currently on-going.

Genotypic analysis among Iranian isolates of *Cercospora beticola*

M. SAFFARIAN ABBAS ZADEH (1), S. Abbasi (2), B. Mahmoudi (3), R. Farokhi Nejad (1)
(1) Chamran University, Ahvaz, Khozestan, Iran; (2) Razi University, Kermanshah, Kermanshah, Iran; (3) Sugar Beet Seed Institute, Karaj, Tehran, Iran
Phytopathology 98:S138

Cercospora beticola is the causal agent of cercospora leaf spot on sugar beet and has a large negative impact on the yield and quality of sugar beet production worldwide. In this study, genotypic analysis among twenty four isolates of *Cercospora beticola* from different regions of Iran was investigated using restriction fragment length polymorphism of the internal transcribed spacer (ITS-RFLP) and RAPD-PCR analyses. Fourteen decanucleotide primers were selected for RAPD analysis. The results of RAPD revealed a wide DNA polymorphism between Iranian isolates of *C. beticola* and clustered all isolates into nine groups. There was a clear relationship between cluster grouping and geographical origin. The restriction pattern of internal transcribed spacer of rDNA (ITS1-5.8-ITS4) was investigated by using three restriction endonuleases *Eco*R1, *Taq*1 and *Busr*1. The undigested DNA fragment length of all isolates was estimated 550bp and no rDNA polymorphism was observed after digestion with endonuleases *Eco*R1 (280, 270 bp), *Taq*1 (330 bp) and *Busr*1 (240, 220, 90 bp). According to these results, RAPD marker is suitable for showing the highest level of genetic variation. On the other hand, ITS-RFLP shows the highest level of similarity and confirms the identification of *Cercospora* spp. These results represented the first documentation of using ITS-RFLP and RAPD for Genotypic analysis among Iranian isolates of *Cercospora beticola.*

In planta distribution and quantification of Asiatic strain of citrus Huanglongbing pathogen

U. S. SAGARAM (1), S. Tatineni (1), J. Kim (1), N. Wang (2)
(1) Citrus Research and Education Center; (2) Citrus Research and Education Center/Department of Microbiology and Cell Science University of Florida
Phytopathology 98:S138

Huanglongbing is one of the most devastating diseases caused by an uncultured phloem limited bacterium, *Candidatus* Liberibacter spp. In this study, a combination of traditional and real-time PCR targeting the putative DNA polymerase and 16S rDNA sequence, respectively, were used to examine the distribution and movement of the Asiatic strain (Las) of citrus Huanglongbing in the infected citrus tree. We found that Las was distributed in bark tissue, leaf midrib, roots, and different floral and fruit parts, but not in endosperm and embryo, of infected citrus trees. In addition, quantitative real-time PCR was used for quantification of the Las in citrus leaf midribs, roots, periwinkle leaves and psyllids. Quantification analysis of the HLB bacterium indicated that Las concentrations varied widely among different tissues of the citrus tree. A relatively high concentration of Las was observed in fruit peduncles. Our data from greenhouse infected plants indicated that Las was systemically transmitted from infection site to different parts of the plant. Our study also indicated that a minimum bacterial concentration is required for HLB symptom development. Understanding the distribution and movement of the HLB bacterium inside an individual citrus tree is critical for discerning its virulence mechanism and to develop management strategies for HLB.

Survey of huanglongbing (HLB) and citrus canker in the Rio Grande Valley

B. SALAS (1), P. Parker (1)
(1) USDA APHIS, Edinburg, TX 78541-5033
Phytopathology 98:S138

Huanglongbing (HLB) and citrus canker are the two most dreaded diseases for citrus production in the Rio Grande Valley (RGV), Texas. The objective of this study was to detect as early as possible these diseases in the RGV and thus to mitigate the tremendous economic losses that can be inflicted on the citrus industry. Within each one mile square quadrat of the RGV, one to four citrus trees have been strategically selected and used routinely to monitor populations of the Mexican fruit fly (*Anastrepha ludens*). Presently, these trees are also being used as sentinel trees to detect HLB and citrus canker. Before the survey, fruit fly trappers from the Texas Department of Agriculture were trained on the recognition of HLB and citrus canker and, thereafter, asked to collect diseased citrus leaves in addition to their normal duties. During years 2006 and 2007, a total 314 and 1685 citrus leaf samples were collected, respectively. The majority of leaf samples were from grapefruit and less from orange. Regardless of host, most samples were from groves and less from dooryards. Samples were examined in the laboratory for symptoms of HLB and citrus canker. Citrus trees showing symptoms of Zn deficiency, yellowing or lop sided fruit were revisited. Other foliar diseases or insect damage were also recorded. None of the citrus leaves or fruits examined showed diagnostic symptoms of HLB or citrus canker. However, a follow up survey is suggested of trees showing symptoms of Zn deficiency (2006 = 15%; 2007 = 8%), mosaic or yellowing (2006 = 3%; 2007 = 7%), or psyllid attack (2007 = 5%). Greasy spot (*Mycosphaerella citri*), Fe deficiency, and Mg deficiency were the most frequent diseases found in this survey. Close monitoring of HLB and citrus canker throughout the Valley must continue.

Species of *Fusarium* associated with the rhizosphere-soil of *Arundo donax* in Laredo-Texas

B. SALAS (1), D. Flores (1), P. Parker (1)
(1) USDA APHIS, Edinburg, TX 78541-5033
Phytopathology 98:S138

Giant reed (*Arundo donax*), has formed dense thickets and is quickly spreading and expanding in its range along the Rio Grande River in Texas and

in many areas of the southwestern United States. Presently, over 170 miles of the river between Laredo and Del Rio, TX are severely infested with this invasive weed. Giant reed threatens the agro-ecosystems and riparian areas by choking river banks and irrigation canals, consuming excessive amounts of water, displacing native plants, and reducing wildlife habitats. Giant reed is an excellent candidate for biological control, because it has no close relatives in North or South America. To find potential pathogenic microbes for the biological control for this non-native invasive weed, soil samples from the rhizosphere of giant reed growing at five locations in Laredo-Texas were collected. Soil samples were dried at 24°C for 4 days, sieved, and 10 mg of soil particles (325-424 microns) were spread onto Komada's culture plates. Representative colonies were identified to species level based on morphological features. Populations of individual *Fusarium* species were significantly different in the soil samples. *Fusarium solani* was the most abundant species found (range 9,900 to 27,900 cfu/g soil) in all soil samples. Other species found in descending order of abundance included *F. equiseti*, *F. moniliforme* (range: 1,184 to 2,472 cfu/g soil), *F. oxysporum*, and *F. subglutinans*. *F. moniliforme* was also isolated from rotten giant reed stem-segments used for propagation. Pathogenicity tests of isolates of all *Fusarium* species are in progress. Pathogenic isolates may be potential candidates for the biological control of giant reed.

Affect of crop residue on colonization and survival of *Phoma sclerotioides*, the causal agent of brown root rot of alfalfa

D. A. SAMAC (2), C. Miyamoto (3), J. E. Larsen (3), L. Atkinson (1), C. R. Hollingsworth (1), C. D. Motteberg (1)
(1) UMN Crookston, NW Research and Outreach Center, Crookston, MN, USA; (2) USDA ARS, St. Paul, MN, USA; (3) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S139

Phoma sclerotioides causes brown root rot (BRR) of alfalfa, and root rot of other perennial legumes and some winter hardy grasses. It can survive as a saprophyte on crop debris so crop residues that support the fungus may increase inocula levels. Current management of BRR is based on crop rotation with spring-sown small grains. We grew eight crop species in the greenhouse in infested field soil, over wintered the pots outside at two locations (St. Paul and Crookston, MN), then measured *P. sclerotioides* density in soil using a quantitative PCR assay. In both years, density of the pathogen was highest in soil from pots with corn, soybean and canola. Density of the pathogen following alfalfa, winter wheat, spring wheat, and oat was similar to the fallow treatment. In addition, we measured colonization of stems, leaves, and roots of the eight crops plus hairy vetch and winter rye under controlled conditions. The origin of the isolate, whether from alfalfa, perennial rye or winter wheat, did not affect colonization. Overall, leaf material from spring wheat, winter wheat, winter rye, and corn; or canola roots supported high levels of colonization while soybean roots supported the lowest colonization. Results suggest that rotation to corn, soybean or canola will not reduce pathogen density on residues for subsequent host plant cultivation. Although colonization of spring wheat leaves was high, results suggest that this does not increase pathogen density in soil.

Powdery mildew of onion caused by Leveillula taurica and the possible epidemiological role of alternative hosts in Idaho and Oregon

R. SAMPANGI (1)
(1) University of Idaho
Phytopathology 98:S139

Powdery mildew of onion (Allium cepa) caused by Leveillula taurica (anamorph =Oidiopsis sicula) is a relatively new disease in the Treasure Valley of southwest Idaho and eastern Oregon. Foliar symptoms include effuse, whitish mycelial growth and circular to oblong, chlorotic to necrotic lesions. L. taurica produces internal mycelium with conidiophores emerging through stomata, and dimorphic (lanceolate and cylindrical) conidia. The teleomorph was not observed on onion. Disease was apparent only on mature plants at the end of growing season. Necrotic leaf spots caused by powdery mildew are often similar to and could be confused with those caused by Iris yellow spot virus which is endemic in this region. Incidence of powdery mildew in commercial onion varieties (in experimental plots) was documented on 3 of 54 varieties during 2006, and on 21 out of 59 varieties in 2007. Search for sources of primary inoculum revealed the following plants infected with L. taurica: Cleome hassleriana, C. lutea and C. serrulata (Capparaceae) and Sphaeralcea grossulariifolia, S. parvifolia and S. coccinea (Malvaceae). Both conidial and teleomorph states of L. taurica were observed on these hosts. Although currently there is no evidence of yield losses in onion due to powdery mildew, increasing frequency of disease incidence and the presence of multiple alternative hosts of the pathogen in the region point to the potential for this disease to become economically important on onion and possibly other crops.

Virulence enhancement of Fusarium oxysporum, a strategy for biocontrol of parasitic weeds?

D. C. SANDS (3), A. L. Pilgeram (3), M. Vurro (1), J. P. Sands (2)
(1) I.S.P.A., Bari, Italy; (2) JEDC LLC; (3) Montana State University, Bozeman, MT, USA
Phytopathology 98:S139

Both witchweed (Striga spp.) and broomrape (Orobanche spp.) are difficult to manage because of prolific seed production and obligate physiological attachment to the host plant. Chemical control is hampered by the sensitivity of the host plant. Strains of Fusarium oxysporum, which have been proposed as mycoherbicides, are host-specific for their target weeds. Strains specific to Striga or to Orobanche have been isolated and evaluated for control of these devastating weeds. However, more efficacious control is required. Biological control is greatly improved by virulence enhancement of the pathogen through amino acid or toxin excretion. Additionally, innovative strategies for cost-effective dissemination such as a carrier seed and for reduction of the large seed bank, through production of strigol-like factors to break seed dormancy, will further increase the effectiveness of biological control. As Striga and Orobanche primarily limit crop production in Africa and the Middle East, the focus hinges on international collaboration.

Influence of chile pepper heat level on root and fruit infection by *Phytophthora capsici*

S. SANOGO (2), M. Tahboub (3), P. W. Bosland (2), L. Murray (1)
(1) Kansas State University; (2) New Mexico State University; (3) Texas A&M
Phytopathology 98:S139

Field observations by chile pepper (*Capsicum annuum*) producers in New Mexico indicate that symptoms of Phytophthora blight (caused by *Phytophthora capsici*) develop slower and its incidence is less in hot than in non-hot chile pepper cultivars. This study was conducted to provide a systematic assessment of the relationship of chile pepper heat level to chile pepper response to *P. capsici*. Three hot (TAM-Jalapeño, Cayenne, and XX-Hot) and two low-heat (NuMex Joe E. Parker and New Mexico 6-4) chile pepper cultivars were inoculated at the 6- to 8-leaf stage with zoospores of *P. capsici*. Additionally, detached mature green fruit from three hot (TAM-Jalapeño, Cayenne, and XX-Hot) and one low-heat (AZ-20) chile pepper cultivars were inoculated with mycelium plugs of *P. capsici*. When plant roots were inoculated, Phytophthora blight was slowest to develop on TAM-Jalapeño in contrast to all other cultivars. When fruit were inoculated, lesion length ratio and lesion diameter ratio were significantly higher for TAM-Jalapeño fruit than for all other cultivars. Mycelial growth on lesion surfaces was more extensive on fruit of TAM-Jalapeño than on fruit of other cultivars. Results indicate that there is little or no relationship between heat level and chile pepper root and fruit infection by *P. capsici*.

Natural co-infection of chile pepper and tall morning glory by *Verticillium dahliae* and root-knot nematode

S. SANOGO (2), S. Thomas (2), J. Scroeder (2), M. Clary (1)
(1) Border Foods, In, Deming, NM; (2) New Mexico State University, Las Cruces, NM
Phytopathology 98:S139

In August 2007, during routine chile wilt surveys, wilted chile pepper plants with high levels of root galling and displaying vascular discoloration were found in a field in Luna County in southern New Mexico. From field diagnostics, it was concluded that plants with these symptoms were infected by root-knot nematode (*Meloidogyne incognita*) and *Verticillium dahliae*. Field diagnostics were validated with recovery of *M. incognita* from plants with root galling symptoms and recovery of *V. dahliae* from all plated stem segments with vascular discoloration. In another field, tall morning glory (*Ipomea purpurea*) was also found with high root galling and both *V. dahliae* and *M. incognita* were recovered from sampled tall morning glory plants. This is the first report of co-infection of chile pepper and a weed species by *V. dahliae* and root-knot nematode. *V. dahliae* isolates recovered from chile pepper, as well as tall morning glory co-infected with root knot nematode, were tested for pathogenicity under greenhouse conditions by transplanting seedlings of a susceptible chile pepper cultivar in soil infested with conidia. Isolates of *V. dahliae* were equally pathogenic on chile pepper with plant wilting and vascular discoloration in the stem observed within 6 weeks of transplanting. *V. dahliae* was recovered from all symptomatic plants. Co-infection of crops and weeds with *V. dahliae* and root-knot nematode may constitute a serious challenge to wilt management on chile pepper.

Inheritance of resistance to early blight disease in a diploid hybrid *Solanum phureja-S. stenotomum* population after one cycle of recurrent selection

J. H. SANTA CRUZ (1), B. J. Christ (1), K. G. Haynes (2)
(1) The Pennsylvania State University, Department of Plant Pathology, Buckhout Laboratory, University Park, PA 16802, USA; (2) U.S. Department of Agriculture, ARS, Plant Science Institute, Vegetable Laboratory, Beltsville, MD 20705, USA
Phytopathology 98:S140

Early blight of potatoes, caused by *Alternaria solani*, is one of the most important diseases in potato growing areas around the world. In Pennsylvania, early blight is the second most important foliar disease and is managed by protectant fungicides. Heritable early blight resistance was previously identified in a diploid hybrid population of *Solanum phureja-S. stenotomum* (*phu-stn*). The most early blight resistant clones from each *phu-stn* family were randomly intermated to develop a second cycle population. The objectives of this study were to determine if recurrent selection for early blight resistance would be successful in this population. Approximately 268 clones, derived from 72 half-sib *phu-stn* families, were evaluated in a randomized complete block design for resistance to early blight. Area under the disease progress curve (AUDPC) was estimated by calculating the percentage of necrotic lesions and defoliation. Broad-sense heritability for resistance was 0.75, with a 95% confidence interval of 0.69-0.81. Narrow-sense heritability was 0.63 ± 0.29. Although these two estimates of heritability were high in both the first and second cycle populations, no genetic gain in early blight resistance was made with this recurrent selection approach. The second cycle population was more susceptible and later in maturity than the first cycle population.

A survey for Phytophthora diseases in mid-Tennessee nurseries: Identification and characterization

L. SANTAMARIA (1), M. T. Mmbaga (1)
(1) Tennessee State University, Otis Floyd Nursery Research Center, McMinnville, TN, USA
Phytopathology 98:S140

Phytophthora diseases impact trees and shrubs in nursery production and landscape settings, but identification of species in mid-Tennessee nurseries have not been done. A survey of Tennessee nurseries was started in 2006 and results from eight nurseries sampled will be discussed. Samples of plant tissues from symptomatic plants, rhizosphere soil and water from irrigation ponds or creaks were evaluated for Phytophthora. Direct isolation of Phytophthora from plant tissues and baiting system for Phytophthora from soil and irrigation water were used in this survey. A total of 660 samples were processed using Phytophthora semi selective media (PARPH). The pathogens isolated were characterized morphologically and using DNA analysis following standard PCR protocols with universal primers ITS1/ITS4. Results of the first 100 samples DNA sequence analysis (Davis Sequencing, Davis, CA) showed that *Phytophthora* spp. was the major organisms in 37% of the samples. Other fungi isolated included *Pythium* 13%, Uncultured endophytes 9%, *Pestalotiopsis* spp. 6%, *Alternaria* 6%, *Absidia/Heterobasidion* 6%, *Fusarium* spp. 6%, *Phoma* spp. 4%, and 12% miscellaneous genera including *Botryosphaeria*, *Ampelomyces*, *Glomerella*, *Giberella*, *Paraconiothyrium* spp. and *Verticillium*. Most of the *Phytophthora* species were isolated from soil and water (36%), and only 1% was from plant tissue. 28% of *Phytophthora* spp. are unclassified *Phytophthora* according to GenBank information. Several Phytophthora species were often found in one nursery. Example: PC nursery had 4 species, *P. cinnamommi* and *P. cryptogea* associated with Juniper, *P. nicotianae* (Cotton Easter), and unclassified *Phytophthora* spp. from their irrigation water. Another nursery had *Phytophthora* spp., and *P. cryptogea/megasperma*. Species of *Phytophthora* in the irrigation water were different from those isolated from the soil or plant tissue. More intensive sampling is needed to determine the association of pathogens in irrigation water with disease incidence in the irrigated fields. Species in irrigation water have the potential to infect susceptible hosts during irrigation. Some of the other fungi isolated from plant tissue, soil and/or water are known pathogens. Their pathogenicity and role in disease complexes will be evaluated.

An integrative approach to characterizing the cucumber-*Pseudoperonospora cubensis* interaction

E. A. Savory (1), M. TIAN (1), C. Erhardt (1), M. Hausbeck (1), R. Hammerschmidt (1), B. Day (1)
(1) Department of Plant Pathology, Michigan State University, East Lansing, MI, USA
Phytopathology 98:S140

Downy mildew is an important disease of cucurbits (e.g., cucumbers, melons, etc.), caused by the obligate pathogen *Pseudoperonospora cubensis*. Historically, cucumber (*Cucumis sativus*) in the United States has been resistant to downy mildew. However, introduction of a potentially new pathotype of *P. cubensis* has caused devastating losses in recent years. Understanding the molecular and genetic interactions between host and pathogen during the infection process will enable us to elucidate the mechanisms of infection and disease progression and allow us to identify potential strategies for breeding downy mildew resistant cultivars. A three-fold approach has been undertaken to identify the genetic basis for enhanced virulence observed in recent field isolates of *P. cubensis*. First, phylogenetic characterization through the use of PCR and sequencing of *P. cubensis* isolates collected throughout the United States will be used to determine the genetic basis for enhanced virulence and to establish a genetic database. This database will allow us to monitor pathogen spread, disease occurrence and adaptation of *P. cubensis* with regard to host and environmental conditions. Second, suppressive subtractive hybridization (SSH) is being used to identify genes which are differentially expressed following infection with *P. cubensis*. These genes will be sequenced and their expression confirmed with RT-PCR and northern blots. Finally, the use of laser microdissection, will enable us to isolate single host cells at various stages of pathogen infection. These isolated cells will be used to profile gene expression during pathogen invasion. This integrative approach will provide aframework for determining the basis of pathogenicity and susceptibility in the *P. cubensis*-cucumber interaction which can be used to direct future management and breeding efforts.

Fungicide efficacy in eradicating powdery mildew and reducing cleistothecium formation on grape leaves

A. C. SCHILDER (1), N. L. Rothwell (2), K. L. Powers (2), M. D. Anderson (2)
(1) Department of Plant Pathology, Michigan State University, East Lansing, MI 48824; (2) Northwest Michigan Horticultural Research Station, Michigan State University, Traverse City, MI 49684
Phytopathology 98:S140

Powdery mildew (*Uncinula necator*) is an important disease of grapes in Michigan. To evaluate the ability of fungicides to eradicate existing colonies and reduce cleistothecium formation, the following products were applied to mature 'Pinot noir' vines in Traverse City, MI: JMS Stylet Oil (paraffinic oil), Kaligreen (potassium bicarbonate), Oxidate (hydrogen dioxide), Prev-Am (sodium tetraborohydrate decahydrate), C+G (organic acids), Cuprofix (basic copper sulfate), Sulforix (calcium polysulfide), and Elite (tebuconazole). On 13 September, 2007, treatments were applied at recommended rates to single-vine plots with a backpack sprayer. About 20% of the leaf area on average was covered with powdery mildew at the time of fungicide application. Plots were replicated five times in a randomized complete block design. Spray volume was equivalent to 935 L/ha. Unsprayed vines served as controls. Disease severity was assessed immediately before and 1 and 2 weeks after application on 20 randomly selected leaves per plot. Cleistothecia were counted on 10 randomly selected leaves per plot on 17 October. After 1 week, JMS Stylet Oil had reduced powdery mildew severity the most compared to the untreated control (by 75%), followed by C+G (59%), Sulforix (53%) and Kaligreen (39%). The other fungicides were less effective. After 2 weeks, disease severity in these treatments was reduced by 88%, 82%, 73%, and 79%, respectively, compared to the untreated control. The total number of mature cleistothecia was reduced most by Sulforix (82%), followed by Elite (78%), Kaligreen (70%), and C+G (66%). The results indicate that a late-season fungicide application can reduce foliar powdery mildew severity as well as production of overwintering inoculum.

Influence of carbon source amendments on population density, resource use, and antibiotic phenotypes of soilborne *Streptomyces*

D. C. SCHLATTER (4), A. Fubuh (1), K. Xiao (2), D. Hernandez (3), S. Hobbie (4), L. L. Kinkel (4)
(1) Fort Valley State University, Fort Valley, GA, USA; (2) USDA-ARS Cereal Disease Lab, St. Paul, MN, USA; (3) University of California, Santa Cruz, Santa Cruz, CA, USA; (4) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S140

Disease suppression by soil microbial communities is often a function of antibiotic inhibition and resource competition. These competitive phenotypes are likely to be dependent on available nutrients. This study explores the effects of repeated carbon source inputs on population densities, resource use, and antibiotic inhibitory phenotypes of Streptomyces in soil. High and low doses of glucose, cellulose, and lignin were added weekly to mesocosms of native prairie soil for 9 months. Culturable population densities were estimated for each community. Resource utilization was determined for individual Streptomyces isolates from each treatment using Biolog SF-P2 plates. Antibiotic inhibition profiles for these isolates were determined against a collection of 5 standards. The greatest densities were produced by cellulose and lignin amendments. Communities with high resource doses had Streptomyces with stronger inhibitory phenotypes than low-dose communities. Streptomyces communities with low resource inputs used substrates more efficiently than Streptomyces from high-input communities. These findings

suggest that dense communities with high resource availability select for good inhibitors while low resource communities select for efficient nutrient use. Thus, resource inputs that promote high Streptomyces densities may produce an environment with strong resource competition and better inhibitory phenotypes that would be more likely to suppress pathogens.

Quantification of damages caused by the Asian soybean rust (causal agent *Phakopsora pachyrhizi*) based in soybean (*Glycine max*) physiological components

J. SCHMIDT (2), W. C. Jesus (1), P. A. Paul (3), F. R. do Vale (2)
(1) Federal University of Espirito Santo, Alegre, Espirito Santo, Brazil; (2) Federal University of Viçosa, Viçosa, Minas Gerais, Brazil; (3) The Ohio State University, Wooster, OH, USA
Phytopathology 98:S141

Soybean rust, the most foliar important disease of the soybean in the world, causes substantial yield losses. With the objective of characterizing the relationship among disease severity (X), Area Under Disease Progress Curve (AUDPC), Leaf Area Index (LAI), Health Area Duration (HAD), Health Area Absorption (HAA) and production, three field experiments were carried out during the 2006 to 2008 growing seasons, at the Federal University of Viçosa, Minas Gerais, Brazil, with the cultivars 'Conquista' and 'Vencedora'. Plants were naturally infected by *Phakopsora pachyrhizi*. In order to obtain a disease gradient, plants in different plots were sprayed with a fungicide (tebuconazole) at different stages of plant growth (before, during and after flowering). Disease severity was accessed using a diagrammatic scale on every leaf of three plants sampled weekly (n = 96), and the yield was estimated from 10 plants harvested per plot. The experimental design was randomized complete blocks with eight treatments and four replications, with each plot being 14 m^2. The relationship between the physiological components and yield were linear ($P < 0.01$). LAI, HAD and HAA had significant positive regressions with yield ($R^2 = 0.57$; 0.73 and 0.46, respectively), whereas AUDPC had a significant negative related ($R^2 = 0.71$) with yield. Yield reduction was observed in plots with higher values of AUDPC as well as lower HAD and HAA values, suggesting that soybean rust negatively affects the photosynthetic efficiency of the soybean plants. Project sponsored by CNPq.

Evaluation of resistance to *Phytophthora megasperma* in rootstocks for species of *Prunus*

L. S. SCHMIDT (1), G. T. Browne (1)
(1) USDA-ARS, Davis, CA, USA
Phytopathology 98:S141

Phytophthora megasperma can cause high incidences of tree death following periods of prolonged soil water saturation in almond and other stone fruit orchards in California. Although many new rootstock selections are available for *Prunus* species, relatively few evaluations of resistance to *Phytophthora* spp. have been completed for them. We examined resistance to *P. megasperma* in three rootstocks used widely among almonds and other stone fruits (Nemaguard, Lovell, and Marianna 2624) and twelve additional rootstocks used either less frequently or experimentally for *Prunus* species. The stocks were rooted as hardwood cuttings, grown in 1–liter pots of UC mix, and transplanted into 2-liter pots of UC mix that was either non-infested or artificially infested with *P. megasperma*. There were 5 randomized complete blocks per treatment. Once every 2 weeks the soil was flooded for 48 h. Between periods of flooding the soil was watered as needed and drained freely. Six months after transplanting, the root systems were washed free from soil and rated for severity of crown and root rot. The six plum hybrids and species (Marianna 2624, AC941 [Microbac], Krymsk#86 [Kuban#86], Ishtara, Empyrean#2, and Hiawatha) were resistant to *P. megasperma* (mean 0 to 5% of crown length rotted, 2 to 7% of roots rotted). In contrast, the peaches and peach hybrids, (Lovell, Nemaguard, Cadaman, and Empyrean#1) were moderately susceptible (21 to 37% of crown length rotted; 21 to 28% of roots rotted) as were the peach × almond hybrids (Cornerstone, Floraguard × Alnem, GxN 15 [Garnem], Hansen 536, and Nickels) (26 to 48% of crown length rotted, 20 to 56% of roots rotted). Apparently, the plum parentages offer resistance to *P. megasperma*.

Attenuation of severity of Asian soybean rust with potassium, chloride and minor elements

R. W. SCHNEIDER (1), J. Wang (2), E. P. Mumma (1), C. L. Robertson (1), C. G. Giles (1)
(1) Dept. Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA; (2) School of PESS, Louisiana State University Agricultural Center, Baton Rouge, LA
Phytopathology 98:S141

This field study was conducted to investigate the effects of potassium, chloride and the minor elements boron and manganese on Asian soybean rust (ASR). KCl was broadcast on the soil surface immediately before planting at 60 and 120 pounds per acre. Other plots received equivalent amounts chloride in the form of $CaCl_2$. Mn and B were applied as foliar sprays at R1 at 0.5 and 0.25 pounds per acre, respectively. Sidedress applications of KCl and $CaCl_2$ were made at R1 either as single applications or supplemental applications following the preplant treatments. Results clearly showed that disease initiation was delayed and rate of disease development was reduced in the better treatments. Preplant applications of either KCl or $CaCl_2$ at 60 pounds per acre Cl resulted in the least disease. Sidedress applications, either as the sole source or supplementary source, were significantly less effective than the preplant treatments. Minor element applications were variable in their effects and did not show a significant interaction with the main effects. There were significant yield responses, which were related to disease severity and rate of disease increase. There were no differences in disease severity across all treatments at late R6. Results from this study suggest that ASR may be attenuated, but not controlled, with nutritional supplements. It is possible that rate and number of applications of fungicides may be reduced in conjunction with this cultural control practice.

DNA markers for identification of the bacterial phytopathogens *Clavibacter*, *Erwinia*, *Ralstonia*, and *Xanthomonas*

K. L. SCHNEIDER (1), A. M. Alvarez (2), G. G. Presting (1)
(1) Molecular Biosciences and Bioengineering, University of Hawaii Manoa, Honolulu, HI, USA; (2) Plant and Environmental Protection Sciences, University of Hawaii Manoa, Honolulu, HI, USA
Phytopathology 98:S141

Genomes of *Clavibacter*, *Erwinia*, *Ralstonia* and *Xanthomonas* were examined to identify DNA markers suitable for species identification. Selection criteria for a DNA marker included 1) presence in the four genera as a single copy, 2) a low rate of horizontal gene transfer as assessed by phylogenomic orthologous protein analysis and 3) having greater variablity than the internal transcribed spacer (ITS), found between the 16S and 23S rDNA. Six *Xanthomonas* genomes were examined using a whole genome analysis program (MUMmer) to find conserved regions from which to design primers. A single region contained within the chromosomal replication initiator gene, *dnaA*, met all of the selection criteria. DNA sequences of the ITS and *dnaA* markers were obtained for 120 *Ralstonia*, 24 *Xanthomonas*, 24 *Erwinia* and 24 *Clavibacter* strains. The *dnaA* marker generally provided resolution equal to or greater than the ITS marker, as exemplified by its ability to separate *Ralstonia solanacearum* race 3 biovar 2 from other strains.

Specific immunodetection of *Phytophthora ramorum* and *P. kernoviae*

B. Schoedel (1), F. J. AVILA (1)
(1) Agdia Inc., Elkhart, IN
Phytopathology 98:S141

Phytophthora ramorum is an important pathogen in the USA and Europe; and *P. kernoviae* is causing problems in ornamental plants and trees in Europe. The objective of this project was to develop serological tests for these two pathogens. Mycelium suspensions of *P. ramorum* from Europe and USA isolates; and *P. kernoviae* from Europe were used as antigens to produce monoclonal and polyclonal antibodies. Antibodies were selected for specificity to *P. ramorum* and *P. kernoviae*. The selected monoclonal antibodies do not differentiate between *P. ramorum* and *P. kernoviae*, or among *P. ramorum* isolates from Europe or USA in ELISA format. The polyclonal antibodies recognized antigens of Phytophthora and Pythium species. Other species of the genus Phytophthora and Pythium have been evaluated to confirm the specificity of the monoclonal antibodies. Preliminary results have indicated that monoclonal antibodies can be used in ELISA or lateral flow formats.

Diversity of *Rhizoctonia* species in eastern Washington as determined by AFLP analysis

K. L. SCHROEDER (2), R. C. Flanagan (1), T. C. Paulitz (2)
(1) School of Molecular Biosciences, Washington State University, Pullman, WA; (2) USDA-ARS, Pullman, WA
Phytopathology 98:S141

Rhizoctonia root rot is prevalent on cereals in dryland cropping systems. *R. solani* AG-8 has been considered as the primary pathogen; however, several additional AG groups have been implicated in causing disease. In addition, these groups of *Rhizoctonia* have aggregated distributions, occurring in patches that can be visually distinct. Little is known about the genetic diversity of these fungi. Isolates were collected from two locations in eastern Washington for analysis using amplified fragment length polymorphism (AFLP). At the Cook Agronomy Farm (CAF) near Pullman, WA (annual precipitation of 500 to 600 mm), 28 isolates were selected and at a second location near Ritzville, WA (annual precipitation of 200 to 300 mm), 133 isolates were collected. Using one primer set, 50 polymorphic loci were identified for the CAF population and 52 polymorphic loci for the Ritzville population. Using sequence confirmed reference strains, the majority (18) of

the isolates from CAF were determined to be *R. solani* AG-2-1. Conversely, at the Ritzville location, one of the largest groups of isolates was *R. solani* AG-8 (37 isolates). Within each of these populations, each isolate formed a distinct haplotype. *R. solani* AG-8 is present at low populations or absent in the higher precipitation zone of eastern Washington, and bare patch symptoms are rare. The lack of clonality is surprising, given the aggregated patchy distribution of these pathogens in the field.

Development of *Enterobacter cloacae* on onion plants, and effect of post-harvest curing temperature on development of Enterobacter bulb decay

B. K. SCHROEDER (1), E. Thyren (1), J. L. Sires (1), L. J. du Toit (1)
(1) Washington State University
Phytopathology 98:S142

Enterobacter cloacae was associated with an outbreak of Enterobacter bulb decay of onion bulbs that caused economic losses during the 2004–05 storage season in Washington State. Trials were initiated to assess: 1) survival of *E. cloacae* on onion foliage, and 2) the effect of post-harvest curing temperature on development of Enterobacter bulb decay in storage. A rifampicin-resistant strain of *E. cloacae* was mist-inoculated (10^3 and 10^5 cfu/ml) onto foliage of 5 to 8 week-old plants of the cultivar 'Vaquero' maintained in a humidity chamber. Bacteria were isolated from 5 replicate plants at inoculation, and weekly thereafter to quantify the population of *E. cloacae*. In addition, onion bulbs of 'Redwing' and 'Vaquero' were injected with 5×10^7 cfu *E. cloacae*/bulb, and then heat-cured at 25, 30, 35, or 40°C for 2 or 14 days. Storage temperature was then decreased to 4°C at a rate of 2.5°C/day. Four replicates of 5 bulbs/curing temperature/curing duration were evaluated for symptoms after 1, 2, and 3 months of storage at 4°C. *E. cloacae* populations on inoculated onion leaves remained $>10^3$ cfu/ml. In the storage trial, severity of Enterobacter bulb decay increased with increasing curing temperature for both durations of curing. The research will clarify the ability of *E. cloacae* to maintain populations on onion foliage, the relationship of this to bulb infection, and the impact of post-harvest curing temperature on development of Enterobacter bulb decay in storage.

Detection of a pathogen shift among the pectolytic bacterial pathogens of potato in Washington state

B. K. SCHROEDER (2), M. Such (2), J. L. Sires (2), L. D. Porter (1), D. A. Johnson (2)
(1) USDA, The Vegetable and Forage Crop Research Unit, Prosser, WA; (2) Washington State University, Pullman, WA 99164-6430
Phytopathology 98:S142

Bacterial tuber soft rot, aerial stem rot and blackleg are significant diseases of potatoes in Washington State. These diseases are caused by *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium atrosepticum*, and *Dickeya chrysanthemi*, all characterized by the ability to produce pectolytic enzymes for tissue degradation. Since 2001, surveys of potato fields were conducted in the Columbia Basin of Washington state. Plants exhibiting symptoms of aerial stem rot, blackleg and tuber soft rot were obtained and bacteria were isolated. Of the 289 isolates collected, 72% were *P. c.* subsp. *carotovorum*, 11% were *P. atrosepticum* and 16% were *D. chrysanthemi*. The results for *D. chrysanthemi* are of considerable interest as this is a significant increase from 1% reported in the 1980's, suggesting that a pathogen shift has occurred among the soft rot bacteria in the Columbia Basin. In addition, strains of *D. chrysanthemi* are more aggressive than *P. c.* subsp. *carotovorum* or *P. atrosepticum* and exhibit a wider host range including corn. The increasing presence of *D. chrysanthemi* in the Columbia Basin could have long ranging effects not only on potato, but also on corn production as well.

Preliminary report on the genome project of *Candidatus* Liberibacter asiaticus

E. L. SCHUENZEL (2), A. Sechler (2), T. Brettin (1), C. Detter (1), M. Wolinsky (1), G. Gupta (1), N. W. Schaad (2)
(1) Los Alamos National Laboratories, Los Alamos, NM, USA; (2) USDA, Ft. Detrick, MD, USA
Phytopathology 98:S142

Huanglongbing (HLB) is considered one of the most destructive diseases of Citrus spp. in the world. The unculturable bacterium *Candidatus* Liberibacter asiaticus (Las) is thought to be the causal agent. A genome sequencing project was initiated for Las to facilitate the design of better diagnostic assays. While not possible to obtain DNA of Las from plant exudates, due to contamination from plants, limited growth of Las occurred on solid media and bacterial cells were collected from the second serial transfer on agar media. Identity was established with two RT-PCR assays based on 16s rDNA and *rpo* genes, respectively. Las purity was determined by sequencing 16s rDNA to evaluate prokaryotic contamination and an 18s rDNA-based RT-PCR assay for plant material. The Las DNA was increased using multiple displacement amplification (REPLI-g Qiagen kit) and sent to LANL/JGI for library construction and sequencing. A total of 192 clones were sequenced and compared against the GenBank nucleotide database using blastn. No significant similarities were found, indicating the sequences were from an unknown organism, most likely Las. Approximately 3800 clones were sequenced resulting in 1-2x coverage of the Las genome and a preliminary assembly was constructed.

Limited cultivation of *Candidatus* Liberibacter asiaticus, suspected causal agent of Huanglongbing of citrus

E. L. Schuenzel (2), A. Sechler (2), H. HU (1), P. Cooke (3), E. Postnikova (2), A. Stone (2), W. Schneider (2), V. Damsteegt (2), N. W. Schaad (2)
(1) Chongqing University, Chongqing, China; (2) USDA, Ft. Detrick, MD, USA; (3) USDA, Wyndmoor, PA, USA
Phytopathology 98:S142

Candidatus Liberibacter asiaticus (Las) and L. americanus (Lam), suspected causal agents of Huanglongbing (HLB) disease of citrus have been categorized as unculturable, phloem-limited bacteria based upon in situ electron micrograph images and 16S rDNA sequence of prokaryote DNA associated with diseased tissues. Using a citrus extract supplemental (CES) agar and a microaerophilic environment, we report the growth of bacteria associated with HLB. Isolations were made by streaking extracts of sterilized leaf petioles and veins onto CES agar. Plates were sealed and incubated for 3-4d at 28°C, until colonies were visible with a binocular microscope. The colonies, positive in a Las-specific and Lam-specific RT-PCR assays, were 0.1 mm or less, irregular, and consisted of a coagulum of cells in a tight matrix after 7d. Cells were 0.3 to 0.4 × 0.5 to 0.8 microns with numerous fimbriae; filaments were occasionally observed. Similar masses of cells were observed in infected tissue by SEM. Serial transfers to new media resulted in very small, single colonies after 3-5d on CES agar. Growth in liquid CES medium resulted in extensive bioflims. Final identification of the causal agent of HLB awaits completion of Koch's postulates.

New Pest Advisory Group: Assessing exotic plant pathogens and pests recently introduced or imminently threatening the United States

K. A. SCHWARTZBURG (1), K. E. Colpetzer (1), B. M. Spears (1)
(1) USDA APHIS PPQ Center for Plant Health Science and Technology (CPHST), Raleigh, NC, USA
Phytopathology 98:S142

The New Pest Advisory Group (NPAG) is the scientific body within USDA Plant Protection and Quarantine (PPQ) that quickly assesses and recommends a course of action for plant pests recently introduced or imminently threatening the United States. Exotic plant pests analyzed by NPAG include arthropods, mollusks, nematodes, pathogens, and weeds. NPAG analysts collect relevant information by conducting a literature review and soliciting information from Federal, State, and University personnel who have regulatory and scientific expertise for the particular pathogen or pest. The main functions of NPAG are to: 1) analyze the risk of plant pathogens and pests believed to be recently introduced or imminent threats to the United States, 2) coordinate information sharing and solicit expertise, 3) assemble an ad hoc panel to ensure expert evaluation, and 4) recommend options and actions for PPQ's response to a new plant pathogen or pest.

Effect of nitrogen fertilization on colonization of anthurium leaves by *Xanthomonas axonopodis* pv. *dieffenbachiae*

M. Schwiertz (1), G. D. PECKHAM (2), A. M. Alvarez (2)
(1) HS Mannheim University of Applied Sciences, Mannheim, Germany; (2) University of Hawaii, Honolulu, HI
Phytopathology 98:S142

Anthurium blight, caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Xad), is a disease with major ramifications in the ornamental flower industry. In the absence of plant injury, it is presumed that Xad enters the hydathodes of leaf margins by chemotactic attraction to nutrient exudates. Although the form of nitrogen in fertilizer is thought to alter the composition of guttation fluid, hence, the incidence and severity of disease, this phenomenon has not been evaluated quantitatively. Susceptible and resistant anthurium cultivars, Marian Seefurth and Pink Frost, respectively, were treated with ammonium or nitrate fertilizers before Xad exposure. Visual and electronic disease assessment methods were compared using a transgenic bioluminescent strain of Xad. Guttation volume and composition was assessed and correlated with disease progression. In addition, the infection process was examined using scanning electron microscopy (SEM). Our results support the use of visual assessment of exposed x-ray films as the electronic method was not suited to late time point evaluations of tissue colonization. Ammonium-treated plants were colonized more extensively by Xad and had larger guttation volumes than nitrate-treated plants. These results suggest that nitrate fertilizers may reduce bacterial blight of anthurium by lowering guttation volume and altering composition of vascular fluids.

Detection of *Puccinia pelargonii-zonalis* on greenhouse grown geraniums using a real-time PCR assay

E. A. SCOCCO (1), J. Buck (2), R. Walcott (1)
(1) University of Georgia, Department of Plant Pathology, Athens, GA; (2) University of Georgia, Department of Plant Pathology, Griffin, GA
Phytopathology 98:S143

Geranium rust (*Puccinia pelargonii-zonalis*) is one of many ornamental rusts that can cause devastating epidemics in greenhouses. The purpose of this research was to develop a real-time PCR assay for the rapid detection of *P. pelargonii-zonalis* in greenhouse-grown geraniums. DNA was extracted from urediniospores of three isolates of *P. pelargonii-zonalis* using a liquid nitrogen approach and a Qiagen DNeasy Plant Mini kit. The internal transcribed ribosomal region of each isolate was amplified using ITS1-F and ITS6R2 primers, cloned and sequenced using a TOPO TA cloning kit according to the manufacturer's directions. Using these sequences, BLAST searches were conducted against NCBI GenBank database and sequence regions that showed high degrees of base-pair divergence were selected to design specific primers. The BLAST search resulted in an 89% sequence similarity between geranium rust and other closely related rust species. Based on the sequence data, primers GRF and GRust-R2 were designed and screened for specificity using DNA from 2 plants (daylily and geranium) and 20 fungal species. A real-time Taqman probe (EAS-Probe1) was developed based on the 131 bp amplicon produced by the geranium rust primers. As expected, only DNA from geranium rust isolates was amplified using the newly developed *P. pelargonii-zonalis* PCR assay. The assay displayed a detection threshold of 100pg of template DNA and 10^5 and 10^4 urediniospores/mL. Future experiments will evaluate the ability of the assay to detect the pathogen in greenhouse-grown geranium tissue. Early detection of this pathogen using real-time PCR has the potential to allow the implementation of more timely strategies for disease management in commercial settings.

Host impact on foreign gene integrity in a virus vector

B. L. SEABERG (1), Y. Hsieh (1), H. B. Scholthof (1)
(1) Texas A&M University, College Station, TX, USA
Phytopathology 98:S143

Viruses can be used as vectors for transient expression of foreign genes. However, virus vectors do not maintain stable expression of foreign genes over time due to recombination events, which can be influenced by the host. We aimed to establish if there was a significant difference in the rate of foreign gene retention by a *Tomato bushy stunt virus* (TBSV) vector expressing green fluorescent protein (GFP) in different hosts. To accomplish this, a novel bioassay was developed, whereby RNA transcripts of TBSV-GFP were rub-inoculated onto different plants and at 3 days post inoculation (dpi), these were used as inoculum for cowpea, a local lesion host. Chlorotic lesions at points of virus infection were counted at 4 dpi and then the leaves were exposed to UV light to count green fluorescent foci. These numbers were used to yield a percentage of lesions with viable TBSV-GFP. In this model, GFP retention was 10% in *Nicotiana benthamiana*, 15% in spinach, and 27% in cowpea. This host-dependent stability may be of value to the biotechnology industry when trying to establish stable expression of a foreign protein from a virus vector as some plants may present a more suitable environment than others.

Control of Pythium root rot in a tobacco float system with surfactants

K. W. SEEBOLD (1), E. Dixon (1)
(1) University of Kentucky, Plant Pathology Dept., Lexington, KY, USA
Phytopathology 98:S143

Pythium root rot is the most commonly encountered disease on tobacco transplants produced in float beds, a hydroponic system utilized by the majority of producers in KY and other areas in the U.S. Control recommendations include sanitation and the preventive use of etridiazole; however, adequate sanitary practices are not employed universally and etridiazole, although effective, is costly and can be phytotoxic. Surfactants have been shown to be effective against Oomycete pathogens in hydroponic vegetable and ornamental systems, and could be of value in transplant production to manage Pythium root rot. Five surfactants were applied at 100 ppm to plastic containers containing 1L of water. An untreated control (inoculated and non-inoculated) was included and etriadiazole was applied at 50 ppm to serve as the chemical standard. Treatments were arranged in a completely random design with 3 replications. Four-week old 'KY 14' tobacco seedlings in 4 × 6-cell styrofoam trays were then floated in the containers. Trays were inoculated with *Pythium* one day after chemical treatment, and plants were evaluated for wilting, root necrosis, and phytotoxicity 14 days later. All treatments reduced wilting of tobacco seedlings compared to the untreated control. Tergitol and Naiad at 100 ppm were as effective in reducing root symptoms as etridiazole at 50 ppm. Silwet, SM-9, Tergitol, and Tween-20 caused greater levels of plant damage than etridiazole. The use of a surfactant, Naid, to manage Pythium root rot shows promise. Additional research is needed to identify other effective surfactants, optimal rates and treatment intervals to achieve adequate control of disease.

Host influence on the fatty acid profiles of selected plant-parasitic nematodes

N. S. SEKORA (1), K. K. Lawrence (1), E. van Santen (1), J. A. McInroy (1)
(1) Auburn University, Auburn, AL, USA
Phytopathology 98:S143

Fatty acid methyl ester (FAME) analysis can be used to differentiate *Rotylenchulus reniformis* and *Meloidogyne incognita* from one another with a Mahalanobis (D^2) value of 3.8 ($P < 0.0001$). Fatty acids that are significant to differentiating these nematodes are consistently found in samples containing 250 or more individuals, though a single nematode can be detected. It is hypothesized that the FAME profiles of these two species will vary depending on what host plant they are extracted from. Populations of *R. reniformis* and *M. incognita* were grown on each of the three host plants tomato, cotton, and soybean. At the end of the sixty day growing period, the nematodes were extracted from the soil and roots of each host. Three hundred sixty samples were evaluated by FAME analysis. The resulting data were analyzed with SAS and Sherlock Analysis Software. The maximum Euclidian distance among the three plant hosts was 12, which indicates the fatty acid profiles are very similar. The D^2 distance between cotton and soybean was 16.4, 18.9 between cotton and tomato, and 11.8 between soybean and tomato. These analyses indicate that there is a difference in the expression of fatty acid profiles among different hosts. When compared to the previously derived profiles of these nematodes, the maximum Euclidian distance among these analyses is 15. This indicates that host plants may not significantly impact the fatty acid profiles of plant-parasitic nematodes.

Identification of a type three secretion inhibitor shared by *Dickeya dadantii* and *Yersinia pseudotuberculosis*

D. A. SELIMI (1), Q. Peng (2), A. O. Charkowski (1), C. Yang (2), M. Elofsson (3)
(1) UW Madison, Plant Pathology, Madison, WI, USA; (2) UW Milwaukee, Department of Biological Sciences, Milwaukee, WI, USA; (3) Umea University, Department of Chemistry, Umea, Sweden
Phytopathology 98:S143

Dickeya dadantii (formerly *E. chrysanthemi*) is a soft rot pathogen belonging to the *Enterobacteriaceae*, which includes other important pathogens such *Escherichia* and *Yersinia*. These pathogens use the type three secretion system (T3SS) for the delivery of effectors into the host. Screening inhibitors of the T3SS may provide insight into new strategies for control. Recently, inhibitors of the *Yersinia* TTSS were identified, and we tested four of these to determine if they also inhibit the *D. dadantii* T3SS. The *D. dadantii* T3SS is required for aggregate formation in culture and one of the four compounds, Y1, inhibited aggregate formation at 50 and 100 μM. We used a green fluorescence protein-based fluorescence-activated cell sorter promoter activity assay to assess the effect of Y1 on transcription of *hrpN* and *dspE*, which encode T3SS-secreted proteins and *hrpL*, the sigma factor that activates the T3SS system. The inhibitor eliminated bistable regulation of *hrpN*. Compared to untreated cultures, *hrpN* and *hrpL* transcription was higher in cultures treated with Y1 after 6 hours. Effects varied at 12 and 24 hours with *hrpN* reduced after 12 and marginally reduced at 24 hours ($P = 0.08$), and *hrpL* reduced after 12 hours but increased at 24 hours compared to untreated cultures. The expression of *dspE* was reduced compared to untreated cultures at all three time points. These data suggest that Y1 has a complex effect on transcription, with differing effects on expression of T3SS genes and suggest that a feedback mechanism may be present. These results have implications for understanding bistable populations, and this is the first report of a T3SS inhibitor with efficacy in both animal and plant pathogens.

The carbohydrate esterase gene family in *Phytophthora infestans*

L. A. Seyer (1), R. W. Jones (2), M. D. OSPINA-GIRALDO (1)
(1) Lafayette College, Easton, PA, USA; (2) USDA/ARS, Beltsville, MD, USA
Phytopathology 98:S143

Phytophthora infestans is an oomycete that is known to cause late blight in potato and tomato. This plant pathogen is known to contain carbohydrate esterases (CE) that may play a role in the infection process by targeting and degrading the cell wall. Based on information obtained from the Carbohydrate Active Enzyme (CAZy) database, a wide scale analysis of the *P. infestans* genome (strain T30-4) using BLAST techniques was conducted with the purpose of identifying genes coding for these CE genes. Out of the 14 CE families listed on the database, 53 putative genes belonging to 8 families were identified. Of these putative genes, 16 candidates from 6 families were chosen for further studies based on their BLAST score. Using specific primers, 94% of these sequences could be amplified from 10 different isolates of *P. infestans*. Genes from families 1, 2, 5, and 11, were present in the highest

number (eight) of isolates. Because our BLAST analysis also revealed that strain T30-4 contains four copies of CE Family 5 (the "cutinase" family, which has been suggested to play a role in pathogenicity), we attempted to amplify these sequences from the available *P. infestans* isolates. PCR amplicons, approximately 700 bp in length, were obtained and subsequently cloned. Multiple clones were sequenced and a phylogenetic analysis was conducted. Results from these analyses are presented and discussed.

Baseline sensitivity of *Fusicladium effusum* to azoxystrobin and *in vitro* toxicity of the alternative oxidase inhibitor, salicylhydroxamic acid (SHAM)

M. SEYRAN (1), K. L. Stevenson (1), T. B. Brenneman (1)
(1) Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton 31793
Phytopathology 98:S144

Pecan production in the southeastern U.S. relies on effective fungicides to control pecan scab, caused by *F. effusum*. Several quinone outside inhibitors (QoIs) are registered for pecan scab control and sensitivity monitoring is important due to the high level of resistance risk. Baseline population sensitivity profiles were constructed with monoconidial isolates of *F. effusum* obtained from three orchards in Georgia with no known fungicide history. Isolates were tested for sensitivity to azoxystrobin using a mycelial growth assay in liquid medium amended with azoxystrobin in microtiter plates, with and without the addition of 100 µg/ml SHAM to suppress alternative oxidase. The 50% effective dose (EC_{50}) values without SHAM ranged from 0.002 to 0.182 µg/ml with a median of 0.02 µg/ml (n = 67) and were log-normally distributed (Prob < W = 0.47). However, when SHAM was added to the medium, EC_{50} values could be estimated from only 25 isolates due to lack of growth or inconsistent dose response to the fungicide. The EC_{50} values of azoxystrobin with SHAM were not distributed log-normally (Prob < W = 0.003). The EC_{50} values of azoxystrobin with SHAM ranged from 0.02 to 0.76 µg/ml with a median of 0.04 µg/ml (n = 25). The sensitivity profile obtained for azoxystrobin without SHAM will serve as a baseline for future fungicide sensitivity monitoring in commercial pecan orchards.

Analysis of survey data for the incidence of white mold in snap bean

D. A. SHAH (1), H. R. Dillard (1)
(1) NYSAES, Geneva, NY, USA
Phytopathology 98:S144

Commercial snap bean fields were surveyed across western New York and in Pennsylvania for the presence of white mold in 2006 and 2007. The goal was to collect data on easily measured variables with the aim of mining the resulting data set for associations between these variables and white mold incidence. Some 1111 observations were made in over 250 fields or plots over the course of the season. White mold incidence was highly skewed, symptoms being absent in the majority of surveyed fields but with a few fields in which there were more than 20% of plants symptomatic. Incidence data were recoded as presence (1) or absence (0) and the resultant binary data explored through piecharts, histograms, scatterplots, coplots, and loess smoothing. These exploratory techniques indicated that white mold presence or absence just before harvest was associated with the degree of canopy closure to a large extent and was somewhat moderated by row orientation (north-south or east-west). Soil moisture, an important component of white mold epidemiology, was highly variable over time in any particular field, and was not a good candidate explanatory variable. Via logistic regression analysis, we found that the odds of white mold increased as the open distance between rows (canopy closure) decreased, as measured before harvest. Also, the odds of white mold in rows oriented north-south was 2.5 times higher than the odds of white mold in rows oriented east-west. The quantitative estimates of white mold presence or absence will be used in the development of decision tools for predicting the risk of this disease to snap bean growers.

Associations between *Cucumber mosaic virus* incidence and aphid dispersal activity in snap bean in New York

D. A. SHAH (1), B. A. Nault (1), H. R. Dillard (1)
(1) NYSAES, Geneva, NY, USA
Phytopathology 98:S144

Virus epidemics in snap bean became noticeably more severe coincidentally at the same time the soybean aphid (*A. glycines*) was introduced into New York (NY) in 2001. Surveys, field and greenhouse studies have so far indicated that the aphid-transmitted *Cucumber mosaic virus* (CMV) is a major component of the virus complex affecting snap bean in NY, and a likely contributor to the observed yield losses over the last several years. During 2002 – 2006 water pan traps were used to sample alate aphids within commercial snap bean fields. Trapped aphids were identified to species by Dr. R. Eckel (RVWE Consulting, Frenchtown, NJ). Plants were sampled and assayed for CMV by DAS-ELISA. Generalized linear mixed models were used to identify associations between CMV incidence and mean cumulative trap counts of different aphid species. Because of the implicated relevance of *A. glycines* to virus epidemics in snap bean, an indicator variable was used to obtain separate parameter estimates for the situations (i) *A. glycines* present in traps, (ii) no *A. glycines* were trapped. When *A. glycines* was absent, CMV incidence was associated with cumulative counts of *Acyrthosiphum pisum* and *Therioaphis trifolii*. However, when *A. glycines* was present, CMV incidence was associated with cumulative counts of *A. glycines* and *T. trifolii*. According to fitted models, *A. glycines* was the species most strongly associated with CMV incidence, with rapid increases in CMV incidence at relatively low trap counts. The model results are consistent with field observations of earlier (with respect to crop growth stage), widespread and more severe symptoms of CMV infection when *A. glycines* alates are present.

Distribution of two cucurbits-infecting poleroviruses in China

Q. SHANG (1), H. Xiang (2), C. Han (2), D. Li (2), J. Yu (2)
(1) Beijing University of Agriculture, China Agricultural University, Beijing, China; (2) China Agricultural University, Beijing, China
Phytopathology 98:S144

Poleroviruses are positive-sense ssRNA plant viruses of which *Cucurbit aphid-borne yellows virus* (CABYV) is the first one reported to infect cultivated cucurbits naturally and to cause a severe disease. The occurrence of CABYV and a new polerovirus tentatively referred to as *Melon aphid-borne yellows virus* (MABYV) were reported in our preliminary research work. From July in 2006 to October in 2007, a large scale survey on the distribution of these two cucurbits-infecting poleroviruses across mainland China was carried out, and cucurbit crops samples with yellowing symptoms were collected from bitter gourd, calabash gourd, cucumber, cushaw, muskmelon, squash, suakwa vegetable sponge, watermelon, wax gourd and honey-dew melon in 21 different provinces including Beijing, Inner Mongolia, Heilongjiang, Liaoning, Tianjin, Shaanxi, Shanxi, Shandong, Hebei, Henan, Gansu, Xinjiang, Hubei, Hunan, Zhejiang, Jiangsu, Shanghai, Guangxi, Guangdong, Yunnan and Sichuan. Totally 190 samples were collected and tested by RT-PCR and sequence analysis. Results showed that 100 samples from all 21 provinces were positive to CABYV and 37 samples only from Inner Mongolia and Beijing were positive to MABYV. Furthermore, co-infection of these two viruses was also investigated.

Development of models for improved prediction of stripe rust epidemics in the U.S. Pacific Northwest

D. SHARMA-POUDYAL (1), X. Chen (2)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, USA; (2) USDA-ARS and Department of Plant Pathology, Washington State University, Pullman, WA, USA
Phytopathology 98:S144

Epidemics of wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, are primarily affected by winter temperatures in the U.S. Pacific Northwest (PNW). Previous models based on temperatures of entire December and January did not provide accurate predictions for some years when the winter month temperatures did not follow the normal pattern. To develop models for more accurate predictions, we conducted regression analyses to determine the effects of average moving temperatures of 10, 20, 30, and 60 days on stripe rust epidemic using temperature and yield loss data of Pullman, WA from 1975 to 2007. In general, the correlation of the lowest average moving temperature of 20 days to yield losses was as good as that of the lowest average moving temperature of 60 days. However, for those years with unusual winter month temperatures, the 20-day model fit the observed value much better than the 60-day model. The new models predict yield losses rather than just disease severities for susceptible cultivars at the flowering stage, making them more useful in the disease management. These models guided us to use temperature factors to determine indices of winter and summer survival of the pathogen in various areas of the PNW, which may lead to geographic mapping of over-wintering and over-summering regions for the pathogen throughout the United States.

The virulence mechanisms of *Xylella fastidiosa* in xylem fluid of citrus and grapevines

X. SHI (2), J. Bi (1), N. Toscano (1), D. Cooksey (2)
(1) Department of Entomology, University of California, Riverside, CA 92521; (2) Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521
Phytopathology 98:S144

Xylella fastidiosa (Xf) is a fastidious, xylem-limited, non-flagellated, insect-transmitted, Gram-negative bacterium that causes many plant diseases, including Pierce's disease (PD). It was investigate that while grapevines are susceptible to the PD strain of Xf, citrus is tolerant or resistant to the PD strain of Xf but may serve as a reservoir for the bacterium. The virulence mechanisms of Xf PD strain between citrus and grapes were investigated by examining the *in vitro* effect of pure xylem fluid from grapefruit, orange,

lemon, and grape on Xf multiplication, aggregation, and attachment of PD strain. The aggregations of large clumps were formed in pure xylem fluid from grapefruit, orange, and lemon trees, whereas a visible thick biofilm was observed in grapevines xylem fluid. Microarray was being applied for analysis of the differential gene expression files of Xf PD strain in differential xylem fluids. Xylem fluid will also be analyzed to determine the chemical compounds or elements that control the virulence of Xf.

Wood-rot disease on cherry trees along Koganei Cherry Street, a national cultural property

J. SHIMIZU (2), K. Fukuda (2), Y. Hayashi (1)
(1) Forest Development Technological Institute (retired), Iidabashi, Chiyoda-ku, Tokyo, Japan; (2) Institute of Natural Environmental Studies, the University of Tokyo, Kashiwa-shi, Chiba, Japan
Phytopathology 98:S145

Koganei Cherry Street, located along Tamagawa canal in Tokyo, has been designated as a national property for its historical value. However, in recent years decline of cherry trees of this street is concerned about shading from surrounding trees and incidence of wood-rot disease. We tried to survey the current condition of wood-rot fungi distribution on the cherry trees and examined the effect of tree size and shading on the fungal occurrence. The survey was conducted five times through 2005 to 2007 for every tree along the street, identifying the fruit bodies of wood-rot fungi, and measuring the tree size and the proportion of the shaded part of the tree crown. During the survey, fruit bodies of wood-rot fungi were found on 51.5% of 666 cherry trees composed of 38 species in 28 genus. Shading did not show significant effect on the occurrence of fruit bodies. On the other hand, tree size had a significant effect and the larger trees suffered from wood-rot disease more frequently than smaller trees. We conclude from these results that as the trees glow larger, the number of pruned and dead stems may increase, causing risk of fungal infection.

Glycoproteins secreted by germinating spores of *Magnaporthe oryzae* determinine the specificity like a suppressor in rice plant-blast interaction

A. Shinjo (1), Y. Okamoto (1), A. Kadoiri (1), T. Arie (1), T. TERAOKA (1)
(1) Tokyo Univ. Agric. & Techn. (TUAT), Fuchu, Tokyo, Japan
Phytopathology 98:S145

In rice plant-blast interaction, the incompatible reaction is not dependent on the cultivar, and also the compatible reaction is not on the pathogenic race. The reaction depends on the combination of the cultivar and the attacking race. Also it is well known that induced resistance and induced acceptability are observed in the interaction. The similar effects to a cultivar were triggered by the fluid of germinating spores in some isolates. And novel glycoproteins, which have a potential activity like a suppressor to induce the acceptability and also to enhance the growth of invasive hyphae, were found in the fluid. Those glycoproteins were fractionated by Concanavalin A affinity chromatography and analyzed by 2 dimensional electrophoresis. Both of the sugar moiety and the peptide chain were involved in the activity. The other fraction in the fluid not-bound to the column had activity to elicit some events associated with the resistant reaction. Those glycoproteins had ability to interact with the mannose-binding rice lectin we found. Glycoproteins secreted by germinating spores of Magnaporthe oryzae determinine the specificity like a suppressor in rice plant-blast interaction.

Identifying the components in *Spl11*-mediated defense pathway and determining the relationship between *Spl11* and other defense signaling genes in rice

G. S. SHIRSEKAR (2), M. E. Vega-Sanchez (2), A. Bordeos (1), M. Baraoidan (1), H. Leung (1), G. Wang (2)
(1) International Rice Research Institute, Manila, Philippines; (2) The Ohio State University, Columbus, OH, USA
Phytopathology 98:S145

Ubiquitination has recently been shown to be involved in programmed cell death (PCD) in plants. *Spl11* encodes an E3 ubiquitin ligase with U-Box and ARM repeat domains which negatively regulates PCD and disease resistance in rice. To identify new components in *Spl11*-mediated cell death pathway, a suppressor screen was performed using the *spl11* mutant GR5717 and EMS as a mutagen. The spontaneous lesion formation observed in GR5717 was found to be completely suppressed in one suppressor, whereas, two other suppressors showed partial suppression. Broad-spectrum resistance in GR5717 was found to be abolished in all the suppressors. To map the genes involved in this suppression phenomenon, three F2 mapping populations were generated using the suppressor lines and *spl11* mutant line TP309spl11. All the three F2 populations show 3:1 ratio of segregation for suppression phenotype to lesion mimic phenotype, indicating a single-gene Mendelian inheritance. Simultaneously F2 populations are also being generated to test for diallelism among different suppressors. Further, genetic analysis to study the relationship between *Spl11* and other defense signaling genes such as *NPR1*, *SGT1*, *RAR1* and *Rac1* has been undertaken. Crosses between *spl11* knockdown or *Spl11* overexpression lines and other defense mutants are being generated. Overall, ubiquitination mediated defense mechanisms will be elucidated through these studies in rice which is the world's most important food crop.

Sporulation on plant roots by *Phytophthora ramorum*

N. SHISHKOFF (1)
(1) USDA/ARS/FDWSRU
Phytopathology 98:S145

Phytophthora ramorum has been shown to infect the roots of many of its foliar hosts. Methods of detecting inoculum in runoff and of quantifying root colonization were tested using *Viburnum tinus*, *Camellia oleifera*, *Quercus prinus*, *Umbellularia californica*, and *Epilobium ciliatum*. Plants grown from seed or cutting in Turface monmorillonite clay granules were inoculated with a sporangial suspension (15 mL per pot at 500 sporangia/mL) and after 24 hours, uprooted, washed, and transplanted to fresh Turface (100 mL volume). Runoff was collected periodically and aliquots plated on selective media to quantify inoculum of *P. ramorum*; at the end of the assay, roots were plated on selective media to determine colonization. In some trials, plant roots were examined at the end of the experiment, and in *Viburnum*, it was easy to see sporulation on root tips. Dissection of *Viburnum* roots revealed embedded chlamydospores. Other host roots, could be heavily pigmented or extremely fine, and signs of the pathogen were not often seen, even in heavily infected material. *P. ramorum* was commonly detected from runoff of all tested plants. In 32 *Viburnum* trials over the course of a year, an average of 41 propagules per pot (4 propagules per mL of runoff) were recovered from runoff from plants seven days after inoculation, with a high of 358 propagules/pot (24 propagules/mL runoff). The significance of such sporulation in the epidemiology of the pathogen needs further study.

A tolerant relative protects tomato against a virulent Verticillium

H. O. SHITTU (1), R. N. Nazar (1), J. E. Robb (1)
(1) University of Guelph, Guelph, Ontario, Canada
Phytopathology 98:S145

Tolerance is a condition in which a host plant exhibits minimal symptoms despite substantial colonization by a potential pathogen. Tolerant plants frequently exhibit interesting properties including protection against virulent pathogens. Craigella tomatoes can be infected with Verticillium dahliae race 1(Vd1) or a nonhost isolate Dvd-E6 (E6) to establish susceptible or tolerant interactions, respectively. The aim of the present study was to determine whether infection with E6 protects tomato against the virulent isolate. Plants were inoculated with E6 or Vd1 alone, or E6 before, mixed with or after Vd1. After 5 or 10 days, the total amount of Verticillium in stems and roots was determined by quantitative PCR. In mixed infections the relative amounts of Vd1 and E6 were assessed by restriction fragment polymorphism, based on the fact that Vd1 has an SpeI site that E6 lacks. When E6 is mixed with Vd1 to infect the plant or E6 inoculation precedes Vd1, Vd1 is almost completely excluded from the root but when Vd1 infects first, E6 is still able to compete on an equal basis. Previous studies suggested that tolerance may be induced by E6 suppression of symptom-related genes in Craigella, raising the possibility that it simultaneously induces tolerance to Vd1; however, this does not seem to be the case. The absence of symptoms following Vd infection of E6 tolerant Craigella appears to result from restricted Vd1 colonization. When Vd1 and E6 are cultured on PDA plates alone or together the growth rates are similar and neither is inhibitory to the other. E6 has no inherent capacity to outgrow or inhibit Vd1; the protective effect apparently requires the interplay of both Verticillium isolates in the plant. Changes in plant gene expression were determined by microarray analyses; interaction-specific differences are discussed. Supported by NSERC and Commonwealth Scholarship to H.O.S.

Identification, pathogenicity and fungicide resistance of fungal contaminants on apple storage room surfaces

P. L. SHOLBERG (1), S. Stokes (1), K. Pohl (1), D. O'Gorman (1)
(1) Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada
Phytopathology 98:S145

Blue mold caused by *Penicillium* spp. is the most important postharvest disease of apples stored in controlled-atmosphere (CA) storages. A survey of CA storage rooms in seven major packinghouses in British Columbia (BC) was conducted in 2005 to determine if CA room surfaces were a source of fungal contamination of stored apples. Room walls and floors were randomly swabbed (10 cm^2 areas) after fruit were removed for packing. Used swabs were vortexed in sterile-distilled-water for 30 s, and 100 µL aliquots were spread over three plates containing potato dextrose agar acidified with lactic acid (LAPDA). Plates were incubated at 20°C for 5 days, when colonies were counted and identified to genus. Twenty representative single-spore isolates of *Penicillium* spp. were identified to species with the polymerase chain reaction (PCR) using the beta-tubulin gene. These isolates were also evaluated for

pathogenicity on apple and resistance to thiabendazole (TBZ). *P. expansum* and *P. solitum* were widespread in CA rooms. *Penicillium cyclopium* was mostly found on fire retardant material. As expected the *P. expansum* isolates were pathogenic whereas the other *Penicillium* spp. varied in pathogenicity. Resistance or tolerance to TBZ was found in seven isolates although none of the *P. expansum* isolates were resistant to TBZ.

Phylogenetics and population biology of a monophyletic group within the *F. solani* species complex that is widely associated with human infections
D. P. SHORT (2), N. Zhang (1), K. O'Donnell (3), D. Geiser (2)
(1) Department of Plant Pathology, Cornell University, Geneva, NY, USA; (2) Department of Plant Pathology, Penn State University, University Park, PA, USA; (3) Microbial Genomics and Bioprocessing Research Unit, Agricultural Research Service, USDA, Peoria, IL, USA
Phytopathology 98:S146

The *F. solani* species complex (FSSC) is a group of ascomycete plant and human pathogens that are common in many environments. The FSSC comprises several dozen phylogenetic species, seven of which correspond to known biological species, but are otherwise morphologically cryptic. We are investigating the phylogenetics, taxonomy and population biology of "Group 2" of the FSSC, a monophyletic group commonly found in the human environment and also frequently in human infections, particularly in the 2006 outbreak of contact lens-associated fungal keratitis. DNA sequence analysis of protein-coding genes suggested that Group 2 may actually comprise more than one phylogenetic species. Using the complete genome sequence of *Nectria haematococca* Mating Population VI (NhMPVI) as a guide, we identified polymorphic microsatellites in intergenic regions and in other portions of the genome useful as genetic markers for studying Group 2 and other members of the FSSC. Ten out of fifteen markers tested were successful in amplifying an expected band in Group 2, "Group 1" (NhMPV, which also commonly infects humans), as well as in the source species NhMPVI. Based on DNA sequence analysis of these regions, microsatellite polymorphism was observed in addition to non-microsatellite insertion/deletion polymorphism and nucleotide substitutions. Inferences about phylogenetic species boundaries, recombination and taxonomy will be presented.

Race characterization of *Pseudomonas savastanoi* pv. *glycinea* in Illinois
S. P. SIEGEL (1), Y. F. Zhao (1), C. A. Bradley (1)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL, USA
Phytopathology 98:S146

Bacterial blight of soybean (*Glycine max*), caused by *Pseudomonas savastanoi* pv. *glycinea* (Psg) is an endemic disease in Illinois. Yield losses of soybean due to Psg have been estimated to be 5 to 15% in Illinois. Researchers have characterized nine physiological races of Psg based on reactions of nine standard soybean differential lines; however, the race distribution for Illinois is not known. In order to characterize the races of Psg in Illinois, soybean leaf samples with symptoms of bacterial blight were collected from fields in 15 counties throughout the state of Illinois in 2007. Bacterial isolations were made from these leaves, and isolates obtained from these samples were confirmed to be Psg using PCR. A total of ninety-two Psg isolates were collected. The soybean differential lines 'Chippewa', 'Harosoy', 'Lindarin', 'Norchief', 'Peking', 'Merit', 'Flambeau', 'Acme', and 'Centennial' were planted in the greenhouse and leaves were inoculated with the collected Psg isolates. Of the ninety-two Psg isolates collected, 63 (68.5%) were determined to be race 4 and 24 (26.1%) were determined to be race 5, while the remaining 5 (5.4%) isolates could not be characterized using this set of nine differential lines.

Gene expression profiling of the infection of yellow potato (*Solanum phureja*) by *Phytophthora infestans*
R. SIERRA (1), A. Vargas (1), A. Bernal (1), S. Restrepo (1)
(1) Universidad de los Andes, Bogota D.C., Colombia
Phytopathology 98:S146

The potato and tomato late blight pathogen, *Phytophthora infestans* has a broad host range within the Solanaceae family, including yellow potato (*Solanum phureja*). The disease caused by *P. infestans* in *S. phureja* is poorly understood and is a major concern in Colombia. Utilizing Serial Analysis of Gene Expression and high-throughput sequencing (SAGE-Solexa), we characterized yellow potato gene expression during infection by *P. infestans*. Four-week-old yellow potato plants were inoculated with *P. infestans* and were collected at 12 and 72 hours post inoculation for RNA extraction. Expressed Sequence Tag (EST) libraries and a normalized library constructed from healthy plant tissue revealed high levels of sequence similarity between *S. phureja* and *S. tuberosum*. We detected differentially expressed genes by comparing inoculated to non-inoculated and resistant to susceptible plants. The discovery and characterization of the proteins mediating this host–pathogen interaction will enable the understanding of the pathosystem and is the key for developing resistant plants.

Control of Asian soybean rust using sequential fungicide applications
E. J. SIKORA (1), D. P. Delaney (1), M. A. Delaney (1), K. S. Lawrence (1), M. Pegues (2)
(1) Auburn University, Auburn, AL, USA; (2) Gulf Coast Research and Extension Center, Fairhope, AL, USA
Phytopathology 98:S146

Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, has been a recurring problem for soybean growers in southwest Alabama. Most growers have adopted a fungicide spray program that consists of two fungicide applications applied at the R2 and R4-5 growth stages. Because of the many fungicides available to growers, we decided to evaluate various fungicide spray programs under the relatively high ASR pressure in this area. The trial was conducted in 2007 in Fairhope, Alabama. The experiment was planted on 7 June with the cultivar DP 7220 RR. Thirteen sequential fungicide spray programs and an unsprayed control were evaluated in a RCB design with four replications. Fungicide treatments were applied as a foliar spray at the R2 growth stage and repeated 21 days later at R4-5. ASR was rated regularly once the disease started to develop. Soybean rust was initially observed in late August at the R5 growth stage in the control plots. All fungicide treatments reduced the severity of ASR compared to the unsprayed control. Severity of ASR was significantly higher for the Quilt-Quilt, Quilt-Laredo and Stratego-Laredo programs compared to all other fungicide programs on 3 October. Severity of ASR was significantly lower for the Headline SBR-Topguard, Quilt-Topguard and Stratego-Topguard programs then all other fungicide programs on 20 October. The trial was harvested on 7 November. All fungicide treatments increased yield compared to the unsprayed control. The Headline SBR-Folicur program produced a higher yield compared to the Stratego-Stratego, Stratego-Laredo and Quilt-Quilt programs.

Evaluation of TOPGUARD for control of Asian soybean rust in Alabama
E. J. SIKORA (1), D. P. Delaney (1), M. A. Delaney (1), K. S. Lawrence (1), M. Pegues (1)
(1) Auburn University, Auburn, AL, USA
Phytopathology 98:S146

Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, has been a recurring problem for soybean growers in the southern United States. Because of the lack of resistant cultivars, growers must rely on timely use of fungicides to manage the disease. The fungicide TOPGUARD (active ingredient flutriafol) is a recently introduced fungicide from Cheminova Inc. In 2006, we evaluated TOPGUARD at various rates and number of applications per season. The trial was conducted at the Gulf Coast Research Center near Fairhope, Alabama. The experiment was planted on 1 June with the cultivar DP 7870 RR. Treatments included TOPGUARD applied at 3.5, 5, 7, 11 or 14 fl oz/A at the R2 growth stage; TOPGUARD applied at 3.5, 5, 7 fl oz/A at the R2 and R5 growth stages; TOPGUARD applied at 7 fl oz/A at R2 followed by 11 fl oz/A at R5; TOPGUARD at 7 fl oz/A tank-mixed with Dithane DF at 21 oz/A applied at R2; and Headline applied at 6 fl oz/A at R2 followed by TOPGUARD at 7 fl oz/A at R5. The 11 spray programs and an unsprayed control were evaluated in a RCB design with four replications. Soybean rust was initially observed in late August at the R5 growth stage in the control plots. Plots were rated for ASR on 13 and 27 September and 9 October. Severity of ASR was significantly higher in the unsprayed control at all three rating dates compared to the fungicide treatments. There were no significant differences in ASR severity among the fungicide treatments. All fungicide treatments increased yield compared to the unsprayed control, however, there were no significant differences in yield among the fungicide treatments regardless of frequency of application. TOPGUARD was evaluated again in 2007 at the same location with similar treatments and similar results.

Emerging of new species of *Pseudomonas in sensu stricto* affecting beans in Mexico
H. V. SILVA-ROJAS (1), L. X. Zelaya-Molina (2), E. Valadez-Moctezuma (2), L. Cordova-Tellez (1)
(1) Colegio de Postgraduados, Texcoco, Edo. de Mexico, Mexico; (2) Universidad Autonoma Chapingo, Texcoco, Edo. de Mexico, Mexico
Phytopathology 98:S146

In the last years, at least two new specis of *Pseudomonas* causing symptoms similar to those of known bean pathogens were founded in seed production plots in tne central High Valley region, Mexico. The first one corresponded to bacterial strains that caused leaf spots similar to those caused by *Xanothomonas axonopodis* pv. phaseoli. However, the polyphasic identification showed that they belonged to a new species of *Pseudomonas* whose closest relatives are species isolated from water or soil in different parts of the world, and none of them are plant pathogens. The second one corresponded to isolates that produced soft rot in hypocotyls of common dry bean seedlings, and also their polyphasic approach showed that they belonged to a new species of *Pseudomonas* whose closest relatives are the *Pseudomonas putida*

group. Molecular tests demostrated that both species have the type III secretion system, and until now it is unknow which evolutionary changes has occured in the type III effector proteins that contributed to the emergence of these two new species as plant pathogens. In Mexico, there are only reports of three bacterial species as causal agents of bean diseses, but these new species are of great importance because of the losses they will be able to cause in the near future, principally by the risk if seed-borne transmission.

Functional characterization of the *harpin binding protein 1* gene in apple in relation to oxidative stress and fire blight resistance

D. K. SINGH (2), S. N. Maximova (1), T. W. McNellis (2)
(1) The Department of Horticulture, 421 Life Sciences Building, Pennsylvania State University, University Park, PA 16802, USA; (2) The Department of Plant Pathology, 207 Buckhout Laboratory, Pennsylvania State University, University Park, PA 16802, USA
Phytopathology 98:S147

Fire blight is a devastating disease of apple, pear, and other rosaceous plants caused by *Erwinia amylovora* bacteria. During pathogenesis, *E. amylovora* secretes the HrpN (harpin) protein, which is essential for full virulence. The highly conserved harpin binding protein 1 (HrBP1) of plants has been proposed to be a receptor for HrpN. To investigate the function of apple HrBP1 (*Malus* × *domestica* HrBP1, or MdHrBP1), we generated stable *MdHrBP1*-RNAi knock-down apple plants of the Royal Gala variety. Semi-quantitative RT-PCR was used to monitor *MdHrBP1* transcript levels. *MdHrBP1* gene expression was undetectable in *MdHrBP1*-RNAi knock-down plants. *MdHrBP1*-RNAi knock-down plants were regenerated in tissue culture, acclimated to open air, and then established in soil in growth chambers. Under high light (600 μE/m2/s) conditions, the leaves of *MdHrBP1*-RNAi knock-down plants accumulated higher levels of anthocyanin compared to control plants. This result suggests that *MdHrBP1*-RNAi knock-down plants could have a reduced ability to scavenge reactive oxygen species (ROS). Experiments to determine if the *MdHrBP1*-RNAi knock-down plants have reduced ROS scavenging ability are underway. In addition, experiments are in progress to determine whether fire blight resistance is altered in *MdHrBP1*-RNAi knock-down plants. These studies will help us understand the role of MdHrBP1 in abiotic stress responses and in the interaction between apple trees and *E. amylovora*.

Genetic analysis and mapping of tan spot resistance genes using DArT markers

P. K. SINGH (1)
(1) NDSU, Fargo, ND
Phytopathology 98:S147

Tan spot, a major foliar disease of wheat, is caused by an ascomycete *Pyrenophora tritici-repentis*. Presently, eight races of *P. tritici-repentis* have been identified worldwide based on the ability to induce tan necrosis and extensive chlorosis symptoms on a set of wheat differential cultivars. The objectives of this study were to determine and map the genetic control of resistance to spore inoculation and culture filtrate of *P. tritici-repentis* races 2 and 5 in a hexaploid wheat population. F1 and F2 generations and an advance population of recombinant inbred lines (RIL) was developed from a cross between the resistant line ND-735 and the susceptible cultivar Steele-ND. Disease assessment, using culture filtrate and spore suspension, of the segregating generations was done at seedling stage under greenhouse conditions. Diversity Array Technology (DArT) markers were used to map the resistance genes. Genetic analysis of the segregating generations with spore suspension and culture filtrate infiltration indicate that the same single recessive gene located on short arm of chromosome 2B, designated tsr6, controls resistance/insensitivity to chlorosis induced by race 5. A single recessive gene previously designated as tsr1, located on long arm of chromosome 5B, was found to control resistance to tan necrosis induced by spore inoculation and culture filtrate in this population. Results of this study confirm that wheat-*P. tritici-repentis* follows the toxin model of gene-for-gene hypothesis.

Phenology of sooty blotch and flyspeck fungi on apples in Iowa

A. J. SISSON (2), J. C. Batzer (2), K. B. Waxman (1), K. Hemnani (2), M. L. Gleason (2)
(1) Cornell University, Ithaca, NY, USA; (2) Iowa State University, Ames, IA, USA
Phytopathology 98:S147

Sooty blotch and flyspeck (SBFS), a disease complex comprised of more than 60 putative species of fungi, blemishes the surface of apple fruit. Observations suggested that colonies of certain SBFS fungi may appear on apples at characteristic times during the growing season. To test this hypothesis, 10 apples on each of three unsprayed Golden Delicious apple trees in each of 3 Midwest orchards were monitored weekly for the appearance of SBFS colonies in 2006. In 2007, the study was expanded to six locations. Colonies on the apples were marked according to the date of colony appearance. After harvest, apples were stored at 4°C for 3 months, then SBFS colonies were counted and classified by morphology. For a subset of colonies from each morphological category, peels were removed and pressed. Mycelium was scraped from the surface of the cuticle, DNA was extracted, and the ITS and portions of LSU regions of DNA were amplified with SBFS-specific primers ITS1-F and Myc1-R. PCR products were digested with *Hae*III restriction enzyme and fragment patterns were observed with gel electrophoresis. RFLP patterns were compared to a library of patterns for previously identified SBFS species. In 2006 and 2007, *Stomiopeltis* sp. RS1 and RS2 were the first to become visible on fruit. *Dissoconium aciculare* consistently appeared close to harvest, and colonies continued to appear during storage.

Assessment of the role of alfalfa in the spread of *Xylella fastidiosa* in California

M. SISTERSON (1), K. Daane (2), S. Thammiraju (2), R. Groves (3)
(1) USDA-ARS, Parlier, CA, USA; (2) University of California, Berkeley, CA, USA; (3) University of Wisconsin, Madison, WI, USA
Phytopathology 98:S147

Grape and almond are planted on a large scale throughout much of California. Both crops are susceptible to diseases caused by *Xylella fastidiosa*. Within California's Central Valley, the green sharpshooter (*Draeculacephala minerva*) is a key vector. This insect is often found in large numbers in cultivated alfalfa fields and alfalfa is a known host of *X. fastidiosa*. We conducted studies to assess the potential for alfalfa to serve as a source of *X. fastidiosa* inoculum or as a source of vectors. Analysis of Geographic Information Systems maps indicates high overlap in the distribution of grapes and almond with alfalfa in Fresno, Kern, and Tulare counties. Within these counties, monitoring of green sharpshooter populations in alfalfa fields determined that green sharpshooter abundance is typically highest on weedy-field margins. In addition, screening of field collected alfalfa, using standard PCR methods, determined that incidence of *X. fastidiosa* in alfalfa is typically low. Thus, our results suggest that alfalfa fields may act as an important source of the vector but current evidence suggests a minor role for alfalfa as a source of inoculum.

Host range of *Phakopsora pachyrhizi*, the causal agent of soybean rust

T. L. SLAMINKO (2), G. L. Hartman (1)
(1) USDA-ARS, Urbana, IL, USA; (2) University of Illinois, Urbana-Champaign, IL, USA
Phytopathology 98:S147

Phakopsora pachyrhizi, the causal organism of soybean rust, was first described in 1903 from leaves of *Glycine max* subsp. *soja*, or wild soybean, in Japan. Since that time, there have been numerous reports of the pathogen on various leguminous species around the world, first in Asia, followed by Australia, Africa, South America, and most recently North America. Prior to its introduction to North America in 2004, *P. pachyrhizi* was reported on approximately 90 host species, but the consolidation of some species decreased the number to 77 legume hosts in 41 genera. After 2004, a greenhouse evaluation of 176 papilionoid legumes inoculated with a mixture of four international isolates of *P. pachyrhizi* in Ft. Detrick, MD greatly expanded the known host range by 65 species and 12 genera, and a subsequent field evaluation added five species and two subspecies. Lesion type and degree of sporulation varied by host, and several hosts had as much sporulation as susceptible soybean types. *P. pachyrhizi* is currently reported to occur on approximately 150 species in 53 genera of the legume family Fabaceae. The host species all belong to a monophyletic group within the Papilionoideae subfamily. Approximately 120 of the known hosts of *P. pachyrhizi* grow in North America and may play a role in the epidemiology of the disease as overwintering hosts or sources of inoculum to soybean.

Polysaccharide benefits dry storage survival of the biocontrol agent *Pseudomonas fluorescens* S11:P:12 effective against several maladies of stored potatoes

P. J. SLININGER (1), C. A. Dunlap (1), D. A. Schisler (1)
(1) National Center for Agricultural Utilization Research, Peoria, IL, USA
Phytopathology 98:S147

Pseudomonas fluorescens S11:P:12 (NRRL B-21133) is a biological control agent able to suppress several storage maladies of potatoes including sprouting, Fusarium dry rot incited by *Gibberella pulicaris*, pink rot incited by *Phytophthora erythroseptica*, and late blight incited by *Phytophthora infestans* US-8 mating type A2. One characteristic of this strain is that it produces a polysaccharide during liquid cultivation, and our objective here was to determine if this product had a role in the bio-control process. First, the polysaccharide was isolated, purified and identified as marginalan, which in cultures accumulated to about 3.3 g/L (defined as 1X concentration). The bioactivity of isolated marginalan applied alone and in combination with washed cells of *P. fluorescens* (2×10^{10} cells/mL buffer) was tested at the 0,

1/3 X, 1 X, and 2X levels in wounded potato assays of dry rot suppressiveness and potato eye core assays of sprout inhibition. Since the formulation and storage of a dry biocontrol agent is desirable for commercial use, the impact of marginalan on cell survival during drying and storage was also examined when washed bacteria formulated at 0, 1/3 X, 1 X, and 2X polysaccharide were applied under two conditions: either in 2 mL/g HyFlo then dried 24 h with airflow at 50–60% relative humidity; or in 1 micro-L droplets placed in replicate wells of a micro-plate dried 1 h in a biohazard hood. Both the Hyflo and micro-plate dry storage results indicated that marginalan significantly reduced cell death after drying, such that the final stable viable cell density was 2.5 or five orders of magnitude higher (respective to storage condition) than if no marginalan were included with the cells. Marginalan had no significant impact on either disease or sprout suppression, and so its main benefit to biocontrol was viable cell preservation during drying and storage.

Influence of fungicides applied before harvest on postharvest gray mold of table grapes

J. L. SMILANICK (2), M. F. Mansour (2), F. Mlikota Gabler (2), D. A. Margosan (2), J. Hashim-Buckey (1)
(1) U. California, COOP Ext Kern Co., Bakersfield, CA; (2) USDA ARS, Parlier, CA, USA
Phytopathology 98:S148

Thiophanate methyl (THM), iprodione (IPR), cyprodinil (CYP), pyraclostrobin+boscalid (PS/BO), pyrimethanil (PYR), or fenhexamid (FEN) reduced colony size of 4 fungicide-sensitive *Botrytis cinerea* isolates by 50% at 12.4, 2.5, 0.61, 0.29/0.57, 0.26, or 0.17 microg/ml, respectively. THM, IPR, CYP, PS/BO, PYR, or FEN were applied at equivalent of maximum approved rates to detached Thompson Seedless (TS) berries at 600, 500, 270, 59/116, 370, or 290 microg/ml, respectively, 24 or 48 h before or after *B. cinerea* inoculation. Postharvest gray mold after 2 weeks at 15°C was lowest after FEN, followed by PYR, CYP, IPR, PS/PO, or THM. In a Ruby Seedless vineyard, water, IPR, CYP, PS/BO, PYR, or FEN were applied at bunch closure and 2 weeks before harvest and decay was assessed after 1 month at 1°C. Only FEN reduced it significantly, from 15.0% among water-treated grapes to 5.9%. Fungicide residues were about 1/3 USEPA maximums. The following were applied in a TS vineyard at flowering, bunch closure, onset of verasion, and 2 weeks before harvest: 1) water; 2) FEN, PS/BO, THM, then IPR; 3) THM, IPR, PS/BO, then FEN; or 4) IPR+FEN, PS/BO+IPR, IPR, then FEN+PS/BO. Postharvest decay after 7 weeks at 0°C was 5.5, 1.9, 1.3, or 0.7%, respectively. In San Joaquin Valley vineyards, isolates resistant to these fungicides occurred, although FEN resistance was rare.

Analyses of selected parasitism genes of the root-knot nematode *Meloidogyne incognita* in *Arabidopsis thaliana*

A. D. SMITH (2), B. Xue (2), G. Huang (3), T. J. Baum (1), R. S. Hussey (3), E. L. Davis (2)
(1) Iowa State University, Ames, IA, USA; (2) North Carolina State University, Raleigh, NC, USA; (3) University of Georgia, Athens, GA, USA
Phytopathology 98:S148

The southern root-knot nematode, *Meloidogyne incognita*, is an important obligate plant parasite of multiple crops species with evolutionary adaptations such as a hollow, protrusible stylet and esophageal gland secretory cells that enable successful invasion and parasitism of host plant roots. Previous studies have identified more than fifty parasitism genes that encode proteins synthesized in root-knot nematode esophageal gland cells to be secreted from the stylet into plant tissues to promote parasitism. Many of the parasitism genes are pioneers without significant homology to genes currently listed in public databases. Functional analyses of eight of the *M. incognita* pioneer parasitism genes designated 2G02, 4D01, 5G05, 6G07, 17H02, 25B10, 1D08B, and 35F03 are under investigation by overexpression in plant tissue and RNA interference (RNAi) gene silencing assays. The effects of constitutive expression of each parasitism gene product with and without the signal peptide are being analyzed in *Arabidopsis thaliana* plants. Changes in visible plant phenotype will be used as a measure of potential parasitism protein function in plant cells. Expression of double-stranded RNA to each *M. incognita* parasitism gene in transformed *A. thaliana* is providing a reciprocal way to analyze for potential RNAi effects on root-knot nematode parasitism of host roots. These strategies could provide knowledge about the function of root-knot nematode parasitism genes and identify parasitism gene products that are essential to host-parasite interaction.

Regression-based modeling of dollar spot epidemics in creeping bentgrass

D. L. SMITH (1), N. R. Walker (1)
(1) Oklahoma State University
Phytopathology 98:S148

Dollar spot of turfgrass is caused by the fungus *Sclerotinia homoeocarpa*. Previously, several weather dependent fundamental prediction models have been developed to predict dollar spot epidemics. These models have been used as disease management tools with limited success. An alternative approach using multiple regression modeling was explored. Number of disease foci and difference in the number of foci from a previous evaluation were determined daily from bentgrass receiving no fungicide. Various site-specific weather variables were recorded using the Oklahoma MESONET. Weather data were transformed to 2-, 3-, or 4-day moving averages. Disease and weather data were subjected to principal components analysis. Weather data were used as independent variables and disease data as dependent variables in regression analysis to explore potential models. As in previous findings, models using 2-day averages of weather variables were marginally better than models using 3- or 4-day averages. Air temperature and relative humidity were highly influential variables. Soil temperature and solar radiation were also identified as important weather variables, but have not been used in previous dollar spot models. Previous models may have had limited success because they over looked these or other variables. More epidemiological research and regression-based modeling will be used to improve predictive models and identify other influential weather variables.

Isolation and functional analysis of novel secreted proteins in *Magnaporthe oryzae*

P. SONGKUMARN (1), S. Chen (1), R. Venu (1), M. Gowda (1), G. Wang (1)
(1) The Ohio State University, Department of Plant Pathology, Columbus, OH 43202
Phytopathology 98:S148

Rice blast disease, caused by fungus *Magnaporthe oryzae*, is one of the most serious diseases of rice. To elucidate the molecular basis of interactions between rice and *M. oryzae* upon infection, we employed a multifaceted approach that combined Robust-Long SAGE (RL-SAGE) and Massively Parallel Signature Sequencing (MPSS) sequencing method and bioinformatics analysis to identify secreted protein genes in *M. oryzae*-infected rice leaves. A total of 217 putative secreted proteins genes of *M. oryzae* were found to express *in planta*. Three primary candidate of secreted protein genes; MG14, MG20, and MG23 were amplified and cloned. Transient expression assay in rice protoplasts and agro-infiltration assay have been performed for functional analysis. We found that MG14 and MG20 induced cell death in rice protoplasts. Moreover, a full-length MG20 significantly induced cell death in rice protoplasts when compared with a truncated version (without signal peptide). Interestingly, agro-infiltration of a full-length MG20, but not truncated version induced cell death in *Nicotiana benthamiana*, suggesting that this protein might be active in apoplast. Protein domain search indicated that MG14 is predicted to be an extracellular membrane-associated protein with a CFEM domain. Also, a zinc binding site was found in MG20. Characterization of these fungal secreted proteins and their targets might provide new insights into the functional interaction of secreted proteins with host proteins.

Modification of culture medium for growth and sporulation of *Phytophthora infestans* (Mont.) de Bary

J. SOPEE (1)
(1) Kasetsart University
Phytopathology 98:S148

Modification of medium for cultivated *Phytophthora infestans* was conducted. Different local grains including mung bean, black and white sesames, red gram, white and black bean, red kidney bean, job's tear, groundnut, wheat, barley, soybean, maize, sunflower, rice, sorghum were used to prepare the media comparing with common media for *P. infestans*. In the media using mung bean, black and white sesames, red gram, white and black bean, red kidney bean, job's tear, groundnut, wheat, barley, soybean, maize, sunflower, rice grain, and sorghum, grain preparation and other ingredients were the same as in the formulation of Rye A agar medium. The preparation was difference in the media using fresh maize grain, fresh sweet pea pod, maize husk, maize cob and glucose was added at 0, 0, 20, and 20 gm/l respectively. Vigorous growth was obtained on the media with red kidney bean and sunflower whereas black bean medium was the best in sporulation.

First report of *Monilinia laxa* causing brown rot on peaches in Brazil

D. C. Souza (2), A. C. Fazza (1), L. A. Camargo (1), L. M. Mio (3), S. S. Angeli (1), L. AMORIM (1)
(1) ESALQ-USP, Piracicaba, SP, Brazil; (2) Ministério da Agricultura, São Paulo, SP, Brazil; (3) UFPR, Curitiba, PR, Brazil
Phytopathology 98:S148

Brown rot is the most important disease of peaches in Brazil, where *Monilinia fructicola* is the unique species reported in peach orchards so far. *M. laxa* occurs in countries that produce and export stone fruits to Brazil, such as Chile and Spain. There was a previous report of *M. laxa* in Brazil in 1952 although this information was never independently confirmed. Among 200 isolates of *Monilinia* spp. from São Paulo and Paraná States, 17 were characterized by culture morphology and PCR multiplex using species-

specific primers. Isolates were cultured on PDA and incubated at 25°C under 12 h light/12 h dark for seven days. Ten isolates presented cream colored colonies with non-lobed margins, sporulation in concentric rings and no 'rosetted' shape. On average, the rate of colony growth was 9.5 mm/day. One isolate from São Paulo presented brown-yellow colored colonies with lobed margins, and 'rosetted' upper surface as described for *M. laxa*. For this isolate, the rate of colony growth was 6.2 mm/day. The remaining six isolates showed intermediate cultural characteristics. DNA of *M. fructicola* (ATCC10) and *M. laxa* (ATCC7 e ATCC8), given by Dr. Marie-José Côté, from Canadian Food Inspection Agency, were used as positive controls in PCR reactions, which was performed with a common reverse primer (MO368-5) and the species-specific forward primers MO368-10R (*M. fructicola*), and laxa-R2 (*M. laxa*). A PCR product of 530-bp expected in the case of *M. fructicola* was observed in 16 isolates, Only one isolate (ESALQ-1) from São Paulo State amplified a 350-bp PCR product corresponding to *M. laxa* standards ATCC7 and ATCC8, confirming the results of the cultural identification.

Fungi associated with frequently prescribed burns of longleaf pine roots
P. SPAINE (1), W. Otrosina (1), M. Sword-Sayer (1), S. Sung (1), J. Haywood (1), D. Leduc (1)
(1) USDA Forest Service
Phytopathology 98:S149

Longleaf pine is a fire adapted species and known for being disease resistant in its native habitat. Restoration of longleaf pine stands has incorporated the use of more stringent management options, including frequent prescribed burn regimes. In this study, longleaf pine plots were biennially burned four times over a 7–8 year period, until 14 years of age. Treatments were either prescribed fire in spring, summer, winter, or no burning (control). An arson wildfire at 14 years of age ended the study; however visual examination of the trees indicated less physiological stress on the biennially burned plots compared to the controls plots. Paired soil cores 20 cm deep were taken from each treatment. One set of cores was partitioned for the assessment of vertical root distribution, and one set of cores was examined for root disease. Roots were extracted from the soil cores, transported to the laboratory, sterilized and plated for observation of root fungi. Insect activity appeared minimal on root surfaces. Some resinosus was present on roots, and the presence of Ophiostomatoid species including Leptographium species were found predominantly in control plot samples.

Regional predictions of potato late blight risk in a GIS incorporating disease resistance profiles, climate change, and risk neighborhoods
A. H. SPARKS (2), R. Raymundo (1), R. Simon (1), G. Forbes (1), K. Garrett (2)
(1) International Potato Center (CIP), Lima, Peru; (2) Kansas State University, Manhattan, KS, USA
Phytopathology 98:S149

National and global estimates of disease risk are becoming increasingly important for research prioritization and policy planning. We are developing potato late blight risk maps for Peru and Uganda, with plans for extending the approach globally. Our analysis addresses the following components. First we determine the potential effects of host resistance and local climate. To support these analyses, we have developed methods for interpolation of meteorological variables to match the requirements of the disease forecasting model. Second, we evaluate the effects of projected climate change on disease risk and associated pesticide use. Third, we develop a new approach for incorporating 'risk neighborhoods' into disease risk estimates, where risk in any given location is also a function of risk in adjacent locations. Predictions based on these approaches will be compared to observed late blight severity in surveys throughout the regions.

Characterizing diversity in the sooty blotch and flyspeck fungal complex in southern Brazil
P. Spolti (2), J. Batzer (1), E. M. Del Ponte (2), R. Valdebenito-Sanhueza (3), M. L. GLEASON (1), K. Hemnani (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA; (2) Depto. de Fitossanidade, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 7712, Porto Alegre, RS 9154000, Brazi; (3) Embrapa Uva e Vinho, Rua Livramento 515, Bento Gonçalves 95700000, Brazil
Phytopathology 98:S149

Reports of the sooty blotch fungal and flyspeck (SBFS) complex on apple have been limited to the north hemisphere. Fungi in the SBFS complex colonize the apple cuticle, creating blemishes and causing significant economic losses in humid, temperate regions. In 2007 three orchards in southern Brazil were surveyed. Colonies on apple fruit were counted and characterized by morphology. Isolates were made on acidified water agar and purified on potato dextrose agar. DNA was extracted from mycelia and the internal transcribed spacer region was amplified and sequenced for 32 isolates. Preliminary evidence suggests the SBFS complex in Brazil includes species within the genus *Peltaster*, *Dissoconium*, and *Zygophiala*. Members of these genera have also been reported to be members of the SBFS complex in North America, Northern Europe and China. Several previously undiscovered species were also isolated and sequenced. The ability of the isolates to reproduce colonies with the same morphology on apple fruit is underway. Morphological characterization of putative species is also being conducted. A worldwide picture of diversity in the SBFS complex is beginning to emerge.

Modeling and visualization of *Alternaria*
E. Spriggs (1), J. Schlecht (1), K. Barnard (1), B. M. PRYOR (1)
(1) University of Arizona, Tucson, AZ, USA
Phytopathology 98:S149

Alternaria are geographically ubiquitous fungi, found in indoor and outdoor environments, easily spread, with many species pathogenic to plants or animals. Correct identification of species is important to determine whether a particular fungus is pathogenic or a saprobe. However, due to their complex microscopic 3-dimensional sporulation structure, *Alternaria* are difficult to correctly identify. Current identification requires observation the fungus as a 2D image through the microscope, estimation of sporulation parameters, frequent change the focal length to see other parts of the sample, and repetition of the procedure many times. This results in considerable time spent at the microscope recording various parameters estimated by eye, which can be subjective and prone to error. In this project, 3D mathematical models of fungi in the genus were developed to assist in taxon identification. These models will be used to quantify fungal structural parameters, automatically identify unknown samples, and serve as a new educational tool for scientists and students. The proposed 3D models incorporate numerous morphological parameters, and by changing their values, can generate instances of each species of the genus. Microscopy images from unknown fungal samples can then be fit to the mathematical models with a computer program, giving an accurate representation and supporting an automatic classification of the given samples. The models and the data can also be displayed in a virtual environment, allowing scientists and students novel opportunity to examine the fungus as manipulable 3-dimensional objects.

Inhibition of *Rhizoctonia solani* by essential oils found in monarda herbage
T. N. Springfield (1), K. D. GWINN (1), B. H. Ownley (1)
(1) University of Tennessee, Knoxville, TN, USA
Phytopathology 98:S149

Soil amendments of monarda herbage that contains high concentrations of essential oils reduce disease losses due to Rhizoctonia damping-off in tomato transplants. The objective of this research was to determine effect of concentration of individual essential oils from *Monarda* spp. on *in vitro* growth of *Rhizoctonia solani*. The experiment was designed as a factorial with 14 essential oils and three concentrations of each oil (0.05, 0.5, and 5.0 mM) in a randomized complete block. Controls were included with no essential oils. The experiment was conducted three times with three replicates per run, for a total of nine replicates. Mycelial plugs of *R. solani* were placed on potato dextrose agar and 10 microliter-aliquots of oil were placed on filter paper in the lid of a Petri plate. The plate was inverted and incubated at room temperature for 3 days. Following incubation, colony radius of *R. solani* was measured at three positions. Fungal growth was calculated as percentage of the control. Percentage data were transformed (arcsine) prior to analysis. The interaction of essential oil and concentration of oil was significant at $P = 0.0001$. At the highest concentration (5.0 mM) of each oil, growth of *R. solani* was significantly less than growth at the lowest concentration (0.05 mM) for all oils except limonene, beta-myrcene, and alpha-pinene. For borynl acetate, carvacrol, linalool, and thymol, growth of the fungus decreased significantly as concentration of the oil increased. Growth of *R. solani* exposed to volatiles of 5.0 mM oil treatments was less than 50% of the untreated control for carvacrol, linalool and thymol. For all other oil treatments (beta-pinene, borneol, cineole, cymene, terpineol, thymoquinone, and gamma-terpinene) growth of *R. solani* with 0.05 and 0.5 mM oil treatments did not differ while growth in the 5 mM treatment ranged from 66 to 87% of the control.

Expression profiling analyses of *Xanthomonas oryzae* pv. *oryzae* mediated by the RaxR response regulator required for AvrXa21 activity
M. SRIARIYANUN (1)
(1) University of California, Davis, CA, USA
Phytopathology 98:S149

Xanthomonas oryzae pv. *oryzae* is the causal agent of bacterial leaf blight, one of the most serious disease in rice. The rice *Xa21* gene encodes a receptor-like kinase that provides immunity against strains of the *X. oryzae* pv. *oryzae* carrying AvrXa21 activity. Eight *rax* genes (required for AvrXa21 activity) from PXO99, a *X. oryzae* pv. *oryzae* Philippine race 6 (PR6) were identified. In contrast to PXO99, which is unable to cause disease in rice lines expressing Xa21, PXO99 strains carrying rax gene mutation lose AvrXa21 activity. RaxH/RaxR, a two component regulatory system (TCS), was shown to

mediate AvrXa21 activity and regulates expression of other *rax* genes but the mechanism has not been described. In this study, we investigated the molecular mechanism of RaxR regulation by using publicly available whole genome Xo array platform. Comparative transcription profiling of PXO99 wild type and mutant strains shows that RaxR controls the expression of several genes including bacterial pathogenesis genes and other TCS. Electrophoresis mobility shift assay confirm that RaxR binds to promoter of RaxR regulated genes. Gene expression analyses provide new opportunities to understand the specificity of the *X. oryzae* pv. *oryzae*–Xa21 interaction and may contribute to identification of AvrXa21.

Novel diagnostic protocol for obtaining high quality sequence from individual *Meloidogyne* spp. juveniles

R. A. STAMLER (1), J. Trojan (1), S. H. Thomas (1), S. F. Hanson (1)
(1) NMSU, Las Cruces, NM, USA
Phytopathology 98:S150

Root-knot nematodes (RKN) (*Meloidogyne* spp.) are the most economically important genus of plant parasitic nematodes worldwide. Being sedentary endoparasites stage II juveniles (J2) are the only commonly recovered stage from soil samples. There are over 80 recognized RKN species worldwide many of which cannot be morphologically distinguished based on J2 characteristics. Current molecular based diagnostic protocols are difficult for many researchers to use. We report a robust method for nematode lysis, PCR amplification, and direct sequencing for identification of individual J2s collected from soil samples. DNA was recovered from mechanically disrupted J2s using a freeze/thaw/vortex protocol, followed by proteinaseK digestion. The 5′ end of the 18S rDNA was amplified and directly sequenced using internal primers to obtain double-stranded sequence for each juvenile. This procedure was successfully used to obtain sequence from multiple *Meloidogyne* species from known green house cultures and unknown field samples. A neighbor-joining tree consisting of sequences obtained in this study and representative Genbank sequences clearly illustrates that this single locus is sufficient for identification of most recognized Meloidogyne species. For species that are indistinguishable based on 18S rDNA sequence, the ability to amplify multiple loci from individual juvenile lysates allows the use of previously described identification protocols for closely related species.

Response of *Alnus tenuifolia* to inoculation with *Valsa melanodiscus*

G. R. STANOSZ (3), L. M. Trummer (5), J. K. Rohrs-Richey (1), G. C. Adams (2), J. T. Worrall (4)
(1) Department of Biology and Institute of Arctic Biology, University of Alaska Fairbanks, AK 99775, USA; (2) Department of Plant Pathology, Michigan State University, East Lansing, MI 48824, USA; (3) Department of Plant Pathology, University of Wisconsin-Madison, WI 53706, USA; (4) USDA Forest Service, Forest Health Management, Gunnison, CO 81230, USA; (5) USDA Forest Service, Forest Health Protection, Anchorage, AK 99503, USA
Phytopathology 98:S150

Valsa melanodiscus (anamorph *Cytospora umbrina*) is associated with cankered and killed alder (*Alnus*) stems in western North America from Colorado to Alaska. The responses of thinleaf alder (*A. tenuifolia*) stems to inoculation with each of two isolates of *V. melanodiscus* were studied in south-central Alaska. At each of two sites, eight stems per isolate were wounded to expose both inner bark and sapwood and inoculated in early May 2007 by placing a colonized agar plug over the wound. Sterile agar plugs were applied to wounded control stems. Sunken, elongated cankers similar to those with which *V. melanodiscus* has been associated resulted on inoculated stems. In contrast, wounded control stems exhibited strong callus production and wound closure. In September 2007, cankers were harvested and lengths were recorded. Mean canker lengths measured externally (data for both isolates pooled) at the two sites were 45 (range 20-156) mm and 73 (range 22-201) mm. Analysis of variance of log transformed data revealed strong support for effect of location (P = 0.04), but not that of isolate (P = 0.12) or interaction (P = 0.20) on canker length. The fungus was reisolated from each inoculated stem, but not from any control stem. The ability of *V. melanodiscus* to cause cankers on thinleaf alder stems is confirmed, and these results support the conclusion that this pathogen is a cause of alder dieback in western North America.

Efficacy of various brassica varieties for the suppression of root knot, ring, and stunt nematodes

K. STEDDOM (3), K. Ong (2), J. Starr (1)
(1) Texas A&M University, College Station, TX, USA; (2) Texas AgriLife Extension Service, Dallas, TX, USA; (3) Texas AgriLife Extension Service, Overton, TX, USA
Phytopathology 98:S150

Plant parasitic nematodes are a serious limit to vegetable production in East Texas. Green manures of “Florida Broadleaf” (FB), “Southern Giant” (SG), and “Bionute White” (BN) mustards, “Garza” radish (GR), “Purple Top” turnip (PT), “Vates” collards (VC), and “Dwarf Blue” kale (DB) were investigated as biofumigants for the suppression of Root Knot (RKN), Sting (SN), and Ring (RN) nematodes in a sweet potato production system. Controls consisted of plots left fallow (FC) or planted to “Elbon” rye (ER). Brassicas were incorporated into the soil 58 days before planting sweet potatoes. Prior to planting sweet potatoes, incorporating GR reduced (P = 0.0043) populations of RKN better than all other treatments and also resulted in fewer (P = 0.0374) RN at harvest of sweet potatoes than FC. The rate of reproduction of RN in GR plots was lower (P = 0.0030) than all other treatments. Total nematode populations, RKN + SN + RN, were lower (P = 0.0091) than FC for BN and VC. Some brassicas are known to be hosts of root knot nematode. Plots with GR had higher populations (P = 0.0034) of root knot nematodes than other treatments prior to incorporation. Yield was not significantly different between any treatments. Stepwise regression indicated that the most important factors for yield were rate of increase of RKN + RN, initial populations of RKN + RN, and RKN populations prior to planting sweet potatoes. This work demonstrates the effectiveness of brassicas as a winter cover crop for vegetable production in the East Texas Region.

Fusarium head blight severity and deoxynivalenol accumulation in wheat spike tissues as a function of *Gibberella zeae* inoculum density

J. M. STEIN (1), L. Osborne (1), K. Glover (1)
(1) Plant Science Dept., South Dakota State University, Brookings, SD 57007
Phytopathology 98:S150

Disease severity in Fusarium head blight (FHB) of wheat is not always correlated with the final level of deoxynivalenol (DON) in the grain. In a previous field study, we found that the latter was regularly associated with the number of *Gibberella zeae* propagules present on the wheat spikes during flowering. This study investigates this putative phenomenon further in an attempt to characterize this relationship. Plants of a FHB susceptible spring wheat line were grown in the greenhouse under supplemental lighting. When the plants were at 50% anthesis, they were spray-inoculated until runoff with a conidial suspension of *G. zeae* at: 0, 100, 500, 1,000, 5,000, 10,000, 50,000, or 100,000 conidia/ml. Disease incidence and severity were rated at 15 days after inoculation and DON concentrations determined for both grain and chaff fractions. Both disease metrics were found to approach their peak level with only 10,000 conidia/ml, indicating that sufficient inoculum was present to cause the maximum level of visually quantifiable symptoms. In contrast, DON levels in both grain and chaff continued to increase with additional inoculum, although the function of this relationship was not consistent between runs of the experiment.

Xylella fastidiosa* isolates from mulberry harbor a 25 kilobase pair plasmid with extensive sequence identity to a plasmid from *Verminephrobacter eiseniae

D. STENGER (1), J. Chen (1)
(1) USDA-ARS, Parlier, CA, USA
Phytopathology 98:S150

A 25 kbp plasmid was present in each of four Californian isolates of *Xylella fastidiosa* from mulberry affected with leaf scorch disease. Fragments of each plasmid were cloned into *E. coli*, sequenced, and assembled into circular contigs of 25,105 bp (pXF-RIV11 and pXF-RIV16) or 24,372 bp (pXF-RIV19 and pXF-RIV25). The four sequences shared greater than 99.8% sequence identity, excluding a 733 bp insertion in pXF-RIV11 and pXF-RIV16. BLAST searches indicated that eight regions (totaling 19,252 bp) shared 75% to 83% nucleotide sequence identity with pVEIS01, a 31 kbp plasmid from the earthworm symbiont *Verminephrobacter eiseniae*. Sequences related to pVEIS01 appear to encode genes involved in DNA transfer, including components of a Type IV secretion system. Regions of the 25 kbp plasmids lacking similarity to pVEIS01 encoded repB/MobA-like and replication initiator genes previously identified on pXF5823 of *X. fastidiosa*. The 733 bp insertion of the two larger plasmids encoded a conjugal transfer protein nearly identical to that encoded by the chromosome of *X. fastidiosa* Temecula 1. These results indicate that mulberry isolates of *X. fastidiosa* harbor plasmids encoding genes associated with DNA transfer and that at some time in the past, ancestors of two unrelated bacterial species occupying distinctly different niches appear to have exchanged genetic material.

A procedure, based on exposure to chlorine gas, for disinfesting watermelon seeds

D. J. Stephens (3), R. W. SCHNEIDER (1), R. Walcott (2), C. E. Johnson (3)
(1) Department of Plant Pathology, Louisiana State University Agricultural Center, Baton Rouge, LA; (2) Department of Plant Pathology, University of Georgia, Athens; (3) School of PESS, Louisiana State University Agricultural Center, Baton Rouge, LA
Phytopathology 98:S150

We developed and tested a procedure to disinfest dry seeds with chlorine gas. The advantage of this procedure as compared to current technology is that

seeds can be treated and then stored without having to be planted because they are not immersed during the treatment process. This procedure, which is amenable to scaling up, should be useful in eliminating troublesome seed borne pathogens, such as *Acidovorax avenae* subsp. *citrulli* on watermelon, and human pathogens, such as enteric bacteria on alfalfa seed used for sprouts, on a large scale. Briefly, seeds were treated in a glass chamber that was fitted with a recirculating fan, pH probe, and a wet/dry bulb thermometer. Chlorine gas was produced by continuously mixing equal volumes of commercial bleach and 0.6M KH_2PO_4 in a beaker within the chamber. Chlorine concentration was monitored with a Kitagawa gas detector system. In this project, watermelon seeds were exposed to about 750 ppm chlorine gas for 3, 6, 9, 12, and 15 hours at which time they were removed from the chamber and tested for germination and contamination with fungi and bacteria. Fungal contamination was assessed by plating seeds on acidified potato dextrose agar, and bacterial contamination was assessed by plating on nutrient agar and incubation of single seeds in nutrient broth. In addition, infestation with *A. avenae* subsp. *citrulli* was assessed with seedling grow-out tests. The optimum time of exposure was 12 hours at which time germination was 98%, and contamination with bacteria and fungi was 0%.

Influence of planting date and tillage on reniform nematode populations in cotton

S. R. STETINA (1), W. T. Pettigrew (1), W. T. Molin (2)
(1) USDA ARS CGPRU, Stoneville, MS, USA; (2) USDA ARS SWSRU, Stoneville, MS, USA
Phytopathology 98:S151

Planting dates and tillage practices were examined for potential impact on reniform nematode (*Rotylenchulus reniformis*) populations in cotton (*Gossypium hirsutum*). A field trial conducted in 2005 and 2006 in Stoneville, MS examined the influence of early (April 1) or normal (May 1) planting dates and conventional or no-till production on early-season root infection by reniform nematode and seasonal changes in the nematode population in the soil. Treatment effects were evaluated on four cultivars (DeltaPine 444 BR, DeltaPine 555 BR, FiberMax 960 BR, and Stoneville 4892 BR) in a randomized complete block design with a split-plot treatment arrangement (main plot is planting date × tillage, subplot is cultivar) and four replications. Soil populations of reniform nematode were measured at planting and harvest, and root infection was assessed 3 to 5 weeks after planting. No consistent interactions between cultivar and either planting date or tillage were detected, so data were averaged across cultivars. Effects on early-season root infection varied from year to year. In 2005, there were no differences in root infection with respect to tillage, but in 2006 more nematodes were found infecting roots in conventionally-tilled plots than in plots that had not been tilled. Planting date effects also were variable. In 2005, more root infection occurred in plots planted at the normal time than in plots planted early. The opposite was true in 2006, when more root infection occurred in plots planted early. However, differences in early-season root infection did not result in changes in reniform nematode soil populations in either year. These findings suggest that modifications to either planting dates or tillage practices will not reduce reniform nematode populations in cotton.

Baseline sensitivity and evidence of resistance to boscalid in *Didymella bryoniae*

K. L. STEVENSON (1), D. B. Langston (1), F. Sanders (1)
(1) Department of Plant Pathology, University of Georgia, Tifton, GA 31793
Phytopathology 98:S151

Gummy stem blight, caused by *Didymella bryoniae*, is one of the most destructive diseases of watermelon in the U.S. Pristine, a formulated mixture of pyraclostrobin and boscalid, has provided effective control of gummy stem blight in the state of Georgia since the development of widespread resistance to azoxystrobin in 2001 and 2002. In 2007, five isolates of *D. bryoniae* were obtained from watermelon fields in Georgia where Pristine failed to control the disease. These isolates were assayed *in vitro* for sensitivity to boscalid. For comparison, 75 isolates of *D. bryoniae* that had been collected in 2002 prior to any boscalid use and maintained in cold storage were recovered and assayed for sensitivity to boscalid. Sensitivity was determined using a mycelial growth assay on potato dextrose agar amended with technical grade boscalid. All 75 of the previously unexposed baseline isolates of *D. bryoniae* were sensitive to boscalid. The range of EC_{50} values was narrow; values ranged from 0.015 mg/liter to 0.106 mg/liter, with a median of 0.055 mg/liter. Relative growth on medium containing 3.0 mg/liter was less than 10% for all 75 isolates. In contrast, all five isolates collected in 2007 were highly resistant to boscalid. EC_{50} values for these five isolates were greater than 3.0 mg/liter, the highest concentration used in the assay. Relative growth on medium containing a boscalid concentration of 3.0 mg/liter was greater than 73% for all five isolates. The frequency of resistance to boscalid in *D. bryoniae* populations in Georgia is currently under investigation.

Biosynthesis of fusaric acid by *Fusarium oxysporum* f. sp. *vasinfectum*

R. D. STIPANOVIC (1), M. H. Wheeler (1), J. Liu (1), L. S. Puckhaber (1), A. A. Bell (1)
(1) USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX, USA
Phytopathology 98:S151

A genetically unique biotype of the Fusarium wilt pathogen was first recognized in wilted and dead Upland cotton (*Gossypium hirsutum*) seedlings in Australia in 1993. Since that time, the disease has spread rapidly with losses >90% in some Australian fields where it was first discovered. Australian biotypes have been inadvertently introduced into the U.S. in at least two shiploads of cottonseed imported into California for dairy feed. These biotypes are a threat to 4–6 million acres of cotton grown on heavy alkaline soils in the U.S. There is limited resistance to the Australian *Fusarium oxysporum* f. sp. *vasinfectum* (F.o.v.) biotype. The Australian biotypes have not yet been found in California cotton fields; however, F.o.v. race 4 was identified for the first time in California in 2000. Race 4 causes severe Fusarium wilt in Pima cultivars (*G. barbadense*). The phytotoxin fusaric acid has been identified within cotton infected with F.o.v. Both the Australian F.o.v. biotype and race 4 produce prodigious quantities of fusaric acid in culture. To determine the importance of this phytotoxin to pathogenicity, we investigated the biosynthesis of fusaric acid using single and doubled labelled 13C-acetate and DL-4-13C-aspartate. Our findings substantiate those of earlier investigators showing that three molecules of acetate are introduced into fusaric acid at carbon atoms 5, 6, 7, 8, 9 and 10, while those from carbons 2, 3, 4 and 11 are derived from aspartate or oxaloacetate.

Integrated control of fire blight with bacterial antagonists and oxytetracycline

V. O. STOCKWELL (1), T. Temple (1), K. B. Johnson (1), J. E. Loper (2)
(1) Oregon State University; (2) USDA-ARS
Phytopathology 98:S151

In the Pacific Northwest of the United States, the antibiotic streptomycin provided excellent control of fire blight until resistant isolates of *Erwinia amylovora* arose. Oxytetracycline (Mycoshield) is now sprayed as an alternative antibiotic. We found that the duration of inhibitory activity of oxytetracycline is similar to that of streptomycin, but oxytetracycline is considerably less effective than streptomycin when the antibiotics are targeted toward sensitive strains. In an effort to improve disease control, we evaluated combinations of biological control agents (*Pseudomonas fluorescens* A506 and *Pantoea agglomerans* C9-1) and oxytetracycline in eight orchard trials inoculated with an antibiotic-sensitive strain of *E. amylovora*. Two bloom sprays of streptomycin or oxytetracycline reduced the incidence of fire blight of blossom clusters by an average of 76% and 42%, respectively compared to water-treated controls. A combination of C9-1 and a protease-deficient A506 provided 42% disease control. An integrated treatment, i.e., one application of biological control agents followed by one application of oxytetracycline, provided 57% control. Biological and chemical methods of fire blight suppression appear to be complementary, and consequently, an integrated strategy consisting of a biological control agent sprayed in early and near full-bloom, followed by an oxytetracycline treatment at late bloom, improved disease control with a reduced number of antibiotic applications.

The virtual irrigation audit: A diagnostic tool for turfgrass disease

L. STOWELL (1), W. Gelernter (1)
(1) PACE Turfgrass Research Institute, San Diego, CA, USA
Phytopathology 98:S151

Virtual irrigation audits can be used to visualize and quantify irrigation distribution so that its relationship to turfgrass disease can be determined. In this study, a Trimble AgGPS 132 sub-meter global positioning system receiver, HGIS Software from StarPal, and a TDS Recon hand-held computer were used in conjunction with irrigation head precipitation specifications to perform virtual irrigation audits in real-time. Turfgrass diseases were mapped and overlaid onto the virtual irrigation audit to help identify the interaction between irrigation design and disease distribution. To confirm the role of irrigation distribution in disease occurrence, soil moisture readings made with Spectrum Technologies' FieldScout TDR meter were also overlaid onto the irrigation audit. Results from Mesa Verde Country Club, Costa Mesa, CA revealed a significant correlation ($r^2 = 0.34$, $P < 0.01$) between precipitation rate (as estimated by the virtual irrigation audit) and reduction in severity of gray leaf spot caused by *Pyricularia grisea* (as measured using a Spectrum Technologies CM1000 chlorophyll meter) on kikuyugrass, *Pennisetum clandestinum*. Presence of the pathogen was confirmed microscopically. Evaluation of relative volumetric soil moisture with the TDR meter confirmed these results, revealing a significant correlation between increasing soil moisture and reduction in gray leaf spot severity ($r^2 = 0.92$, $P < 0.0001$).

Assessing population structure of the most prevalent North American races of *Puccinia graminis* f. sp. *tritici* using molecular markers
S. M. STOXEN (2), L. J. Szabo (1)
(1) USDA-ARS Cereal Disease Laboratory, St. Paul, MN; (2) University of Minnesota, St. Paul, MN
Phytopathology 98:S152

In the first half of the 20th century major epidemics of *Puccinia graminis* f. sp. *tritici* the causal agent of wheat stem rust, led to significant economic loss throughout the central plains of North America. *P. graminis* f. sp. *tritici* is macrocyclic, heteroecious fungus in which common barberry, the alternate host, is required for completion of the sexual life cycle. A major component of the strategy to control *P. graminis* f. sp. *tritici* has been a barberry eradication program in the United States. Without the alternate host *P. graminis* f. sp. *tritici* is predominantly an asexually population based on the repeating cycles of the dikaryotic (n+n) urediniospores. Currently this rust fungus is characterized into races, which are based on avirulence/virulence phenotypes on a standard set of 16 wheat differentials. To test if these races reflect the clonal growth of this fungus or convergent evolution simple sequence repeat (SSR) markers were used. SSR markers had previously been developed from an enriched SSR library and more recently a second set of markers has been developed from the annotated sequence available from the Pgt genome-sequencing project. Preliminary SSR data and previously examined RAPD data suggest that each race cluster forms a distinct clade and represents a single clonal lineage. The SSR markers were then used to examine phylogenetic relationships between races to test if new races arose from recombination or mutation within the existing population or from a new introduction event. Development of a standard set of genetic markers will be important for genotyping isolates of *P. graminis* f. sp. *tritici*.

Sugar beet cultivar selection for storability and rhizomania resistance
C. A. STRAUSBAUGH (3), E. Rearick (1), P. Foote (2), I. A. Eujayl (3)
(1) Amalgamated Research Inc., Twin Falls, ID, USA; (2) Amalgamated Sugar Co.; (3) USDA-ARS NWISRL, Kimberly, ID, USA
Phytopathology 98:S152

Sucrose loss from sugar beet in storage can result from respiration, fungal growth, and the influence of field infection with diseases such as rhizomania caused by *Beet necrotic yellow vein virus* (BNYVV). Studies were initiated in an effort to reduce storage losses and improve resistance to BNYVV through establishing a cultivar selection program. In 2006, 32 commercial sugar beet cultivars were grown near Paul, ID in soil naturally infested with BNYVV. At harvest on 6 Oct, three 8-beet samples from each plot were collected. Two samples were used to establish percent sugar at harvest and the other sample was placed on an indoor pile (set point 2°C) in a randomized complete block design with 4 reps in Paul, ID. On 5 Mar 2007 (150 days in storage), rhizomania ratings for cultivars ranged from 1 to 5.8 (0 = healthy and 9 = dead) and sucrose reduction ranged from 13 to 90%. Root area covered by an undescribed basidiomycete on 1 Feb 2007 ranged from 8 to 81%. Cultivars that retained the most sucrose had resistance to BNYVV, the least fungal growth, and good storability. Sugar beet cultivar selection using an indoor storage facility will likely prove to be an important tool for improving resistance to BNYVV and reducing sucrose losses in storage.

A summary of diagnostics conducted by the USDA-APHIS-PPQ Molecular Diagnostic Laboratory
P. SUDARSHANA (2), R. Shukla (2), G. Abad (2), B. R. Olson (1), M. Palm (2)
(1) Oklahoma State University, Stillwater, OK 74078; (2) USDA-APHIS-PPQ, Beltsville, MD 20705
Phytopathology 98:S152

The new APHIS-PPQ Molecular Diagnostic Laboratory (MDL) was established to conduct operational diagnostics and confirmatory testing for PPQ program pests including *Phytophthora ramorum* (sudden oak death and ramorum blight), *Liberibacter asiasticus* (citrus greening), *Xanthomonas axonopodis* pv. *citri* (Asian citrus canker) and *Globodera pallida* (potato cyst nematode). Since June 2006 more than 500 *P. ramorum* samples from 21 states were received for confirmatory testing. Nearly 70 samples were determined to be positive for *P. ramorum* using morphology for cultures and a combination of conventional and real-time PCR methods for DNA extracted from plant tissue and water or soil bait samples. *P. ramorum* was detected for the first time on *Magnolia virginiana* which is not currently on the SOD host list. In addition to the program pests, MDL provided molecular diagnostic support for a regional domestic survey for exotic pea leaf minor. The MDL also identified and characterized a number of exotic pathogens from port interceptions, postentry quarantine material and other samples in support of resolving international trade issues. Some examples include first report of *Botryosphaeria lutea* association with maple canker; confirmatory tests for laurel wilt (Red bay wilt) pathogen *Raffaelea* sp., first report of sweet orange scab disease caused by *Elsinoe australis* in Uruguay; bacterial strain differentiation in pineapple heart rot disease caused by *Erwinia chrysanthemi*.

Effects of static and variable storage temperatures on the survival and growth of *Escherichia coli* O157:H7 on prewashed bagged lettuce
M. R. SUDARSHANA (2), S. Bandyopadhyay (2), C. Rosa (2), T. V. Suslow (1), L. J. Harris (2)
(1) Department of Plant Sciences, University of California, Davis, CA 95616, USA; (2) Western Institute for Food Safety and Security, University of California, Davis, CA 95616, USA
Phytopathology 98:S152

Bagged leafy greens have been implicated in outbreaks of enterohemolytic *Escherichia coli* O157:H7 (EHEC) infections in humans in the U.S. In several of these outbreaks, most of those who became ill were thousands of miles away from California, where the implicated produce was grown and processed. EHEC are known to multiply on lettuce and we hypothesized that the risk of infection EHEC contamination increases in permissive post-harvest conditions. Therefore, we investigated the effect of temperature on the survival and growth of EHEC on pre-washed, chopped Romaine lettuce (CRL) and grated Iceberg lettuce (GIL), obtained from a regional distributor. Lettuce samples were spot-inoculated with ca. 30,000 cells of an EHEC isolate obtained from human fecal sample from a lettuce-associated outbreak, bagged in polyfilm bags, incubated at various temperatures, and processed at different times for EHEC. On chopped CRL held at 4°C, the EHEC population decreased by approximately two logs after five days. At 20°C, the population increased by one log within 24 hours and remained constant thereafter. In GIL, the EHEC population increased by >1 log after 24 hours and continued to increase throughout the five day study period. Storage at 20°C for 48 hours resulted in deterioration of quality which was more in GIL. To simulate the effects of variable temperatures, a common occurrence during distribution, prepared CRL and GIL were inoculated with EHEC, incubated at 4°C for two days, transferred to 20°C for 6, 12, 18 and 24 hours, and then returned to 4°C for at least 24 hours before enumeration of EHEC populations. In CRL, the EHEC population increased after following six hours of incubation at the initial 20°C interval, where as with GIL, the EHEC population increased more rapidly, within the first six hours.

Soil microbial communities among different cropping sequences and their effect on the occurrence of peanut soilborne pathogens
H. SUDINI (1), R. N. Huettel (1), C. Arias (2), K. L. Bowen (1)
(1) Dept. of Entomology and Plant Pathology, Auburn University, AL 36849; (2) Dept. of Fisheries and Allied Aquaculture, Auburn University, AL 36849
Phytopathology 98:S152

Microbial communities associated with the agro-ecosystems play an important role in the crop health and productivity. Crop rotations can alter the diversity and richness of microbial communities which appear to influence levels of soilborne plant pathogens. In this study, we are investigating the effect of soil microbial communities associated with different peanut rotation sequences on the occurrence of important soilborne pathogens, such as *Sclerotium rolfsii* (causal agent of stem rot of peanut) and mycotoxigenic *Aspergillus flavus*. We have used ARISA (Automated Ribosomal Intergenic Spacer Analysis) to fingerprint fungal microbial communities in a peanut rotation system. The results indicated similarities among fungal ARISA profiles of the same cropping sequence even though the replicated plots are widely spaced. In order to reveal the frequency of occurrence of the soilborne pathogens in the total fungal communities, we extracted DNA from pure cultures of *S. rolfsii* and *A. flavus*. The DNA from these two fungi are being used to monitor their occurrence in each peanut rotation system at three sampling times, pre-plant, pegging, and harvest. The banding patterns corresponding to these soilborne pathogens could aid in determining their frequency of occurrence over different sampling dates as compared to total fungal populations.

DNA based detection of *Epichloë/Neotyphodium* endophytes from host grasses with combined use of FTA card and genera/species specific PCR primers
K. SUGAWARA (1), C. Schardl (2), I. Okabe (1), T. Tsukiboshi (1)
(1) National Institute of Livestock and Grassland Science; (2) University of Kentucky
Phytopathology 98:S152

Festuca-Lolium grasses infected with *Epichloë/Neotyphodium* endophytes have been introduced as forage and/or turf to Japan mainly from the USA and European countries for about a century, but they also have been invading the islands as weeds from various sources and spreading rapidly. For better application and management of those grasses both in agro-ecosystems and

nature, detection and identification of the endophytes are increasingly important in the islands. Using Whatman FTA-PlantSaver Cards and PCR primers designed for rDNA of the fungi, we tried their detection and identification from growing infected plants. Basal parts of fresh, infected plant tillers were crushed on the card with a pestle, air dried, and 2-mm disks from the card was used as PCR templates following the supplier's instruction. With combined use of two different forward primers designed for the rDNA ITS-1 region of the fungi, one designed for *N. occultans* and *N. uncinatum*, and the other for other related species including *N. lolii*, *N. coenophialum* and several *Epichloë* species, together with an universal reverse primer (ITS4), we could amplify the target region from the card and distinguish those two groups. The former group is not toxic to grazing animals, but the latter group contains some toxic species. However, maybe due to low biomass of the endophytic fungi throughout the host tissue, the target was amplified from only about half of the infected samples tested. The simplicity of the DNA preparation and storage method, which requires no major instrumentation or refrigeration, would be a great advantage for related studies, but further improvement to increase the efficiency will be needed.

The race of *Albugo candida* causing disease on perennial pepperweed, *Lepidium latifolium* in Colorado

M. J. SULLIVAN (1), R. T. Zink (1)
(1) USDA-APHIS-PPQ-CPHST
Phytopathology 98:S153

A white rust disease has been identified on the leaves of perennial pepperweed plants across the United States, especially during wet years, but the reports of the efficacy of white rust are quite variable. Eleven races of *Albugo candida* cause white rust on a range of hosts within the Brassicaceae. However, there have not been reports on which reported race causes white rust of perennial pepperweed. The purpose of this project was to determine the race of *Albugo candida* on pepperweed using an established host differential. Diseased leaves of were field collected in Boulder and Fort Lupton Colorado and maintained on perennial pepperweed in the greenhouse and using a detached-leaf method. Four- to five-week-old plants and one-to-two week old seedlings were inoculated with *Albugo candida*. Sporangial suspensions were prepared by agitating pieces of leaves with pustules in distilled water. The suspensions were strained through cheesecloth and adjusted to 1×10^5 sporangia per ml using a hemacytometer. Suspensions were sprayed to run-off onto the adaxial surfaces of leaves using a hand-held spray bottle. Plants were spray inoculated and placed in a dew chamber at 21°C for 24 hours. Plants were removed from the dew chamber and incubated in the greenhouse at 20 to 30°C until symptom development, approximately 10–14 days after inoculation. Results indicate that the race of *Albugo candida* affecting perennial pepperweed is not one of the currently described races and may represent a new race of *Albugo candida*. Determining which natural enemies are already present in the United States and assessing their current and potential impact on the target weed is a logical first step in developing biological control as a viable management option for perennial pepperweed.

Diversity of fungi causing flyspeck-like signs on apple in China

G. Sun (1), R. Zhang (1), H. Li (1), M. L. GLEASON (2)
(1) College of Plant Protection Northwest A&F University, Yangling, Shaanxi, China; (2) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 98:S153

Flyspeck, a common disease of apple in humid areas worldwide, was previously believed to be caused by a single species of fungus, *Schizothyrium pomi*. In 2005 and 2006, Batzer and co-workers reported that the flyspeck complex on apples in the eastern U.S.A. encompassed several morphological types - flyspeck, compact speck, and discrete speck - within ten putative species in five genera. During the fall of 2006, 32 apples with flyspeck-like signs were collected from orchards in Shaanxi, Henan, and Gansu Provinces of China. Pure isolates were obtained from each sample. Based on morphological characters and sequence analysis of the ITS region of ribosomal DNA, 10 isolates were delimited into three species of *Zygophiala: Z. wisconsinensis*, *Zygophiala* sp. 1, and *Zygophiala* sp. 2. Eleven isolates were attributed to two putative species of *Ramularia*. Four isolates were identified as *Dissoconium mali*, and other seven isolates were not classified. Based on the size and density of sclerotium-like bodies on the fruit, samples from which *Zygophiala* spp. were obtained were placed in the flyspeck mycelial type, whereas other samples did not match previously described mycelial types. One to three isolates from each putative species, totaling 13 isolates, were inoculated on apple fruit, and morphology of colonies that developed on inoculated apples was similar to that of the original colonies on the sampled apples. Our research revealed that the FS complex in China encompasses at least seven species in three genera, and confirmed that flyspeck and related colony morphology types are caused by a taxonomically diverse assemblage of fungi.

First report of *Aureobasidium pullulans* causing sooty blotch on apple fruit in China

G. Sun (2), X. R. Zhai (2), R. Zhang (2), M. L. GLEASON (1)
(1) Iowa State University, Ames, IA, USA; (2) Northwest A&F University, Yangling, Shaanxi, China
Phytopathology 98:S153

Sooty blotch and flyspeck (SBFS) is a complex of fungi that blemish the cuticle of apple fruit, causing losses to apple growers throughout moist temperate regions of the world. A new sooty blotch pathogen was found in a 2006 survey of several orchards in Shaanxi, Tibet and Gansu Provinces, China. The colony morphology type on apples was classified as fuliginous. Hyphae were hyaline, sometimes developing into blackish brown, thick-walled chlamydospores. Conidiogenous cells were undifferentiated, and mostly intercalary in hyaline hyphae. Conidia were produced synchronously in dense groups from small denticles, later forming percurrently and adhering in slimy heads. Conidia were hyaline and ellipsoidal, but very variable in shape and size. Morphology of three isolates was consistent with *Aureobasidium pullulans* described by de Hoog. After isolation in pure culture, DNA was extracted and amplified, and ITS sequences were analyzed. Based on results of a BLAST search, alignments were performed, a majority consensus tree was constructed, and the robustness was evaluated by 1000 bootstrap replications. The isolates with *A. pullulans* clustered together with 95% bootstrap support, although there were some differences in their DNA sequences. A modified version of Koch's postulates was performed in incubators. Colonies appearing on inoculated apple fruit were quite similar in morphology to colonies from which the original isolates had been made; colony type was identical, but colony diameter was smaller. *A. pullulans* has been reported to colonize leaves of many plant genera, as well as soil, fresh water, marine estuary sediments, and wood, and to cause human disease. To our knowledge, this is the first report of *A. pullulans* as a member of the apple sooty blotch complex.

First report of *Colletotrichum acutatum* causing ripe rot of grape in China

G. Sun (2), J. Cui (2), R. Zhang (2), M. L. GLEASON (1)
(1) Iowa State University, Ames, IA, USA; (2) Northwest A&F University, Yangling, Shaanxi, China
Phytopathology 98:S153

Ripe rot, caused by several *Colletotrichum* spp., attacks mature grapes as they ripen. It has been found in most regions where grapes are grown. During the summer of 2007, 11 grape samples were collected from Shaanxi Province, China. Morphological traits of two isolates were identical with those of *C. acutatum*. Conidia were fusiform, aseptate and hyaline, and ranged from 8.5 to 16.5 × 2.5 to 4 µm. Slow-growing colonies were pinkish-purple with a faint shading of light mouse grey aerial mycelia, sclerotia and setae were absent, and orange conidial masses had formed. The other isolates were identified as *C. gloeosporioides*. Pathogenicity tests were carried out by wound inoculating six healthy excised individual grapes. Spore suspension (1 × 105 conidia/ml) was obtained from a 2-week-old putative *C. acutatum* culture grown on PDA. After fruits were wounded with a sterile needle, they were dipped in 6 ml spore suspension, then sealed in plastic bags, and incubating in a moist chamber. Ripe rot symptoms were observed on inoculated fruits after 3 days, whereas control fruits inoculated with sterile water did not develop symptoms. Pathogenicity tests were performed three times. Fungi re-isolated from grapes inoculated with putative *C. acutatum* isolates were identical in morphology to the isolates used for inoculation. To our knowledge, this is the first report of *Colletotrichum acutatum* causing ripe rot on grapes in China.

First report of *Dissoconium mali* associated with flyspeck signs on persimmon

G. Sun (2), H. Li (2), R. Zhang (2), M. L. GLEASON (1)
(1) Iowa State University, Ames, IA, USA; (2) Northwest A&F University, Yangling, Shaanxi, China
Phytopathology 98:S153

During October of 2007, persimmon fruit displaying signs of flyspeck were sampled from an orchard near Yangling, Shaanxi Province, China. The sclerotium-like bodies on the surface of the persimmon fruit ranged from 210 to 340 µm diameter at a density of 0.5 to 1.25 structure/mm^2. Pure isolates were obtained from the colonies, followed by sequencing of the ITS region of ribosomal DNA. The ITS sequence was identical to that of *Dissoconium mali* (strain LQ73, GenBank code EF627452), and morphological characteristics of conidiophores and conidia coincided with those of *D. mali*. A modified Koch's postulates procedure was conducted; persimmon fruits inoculated with the isolate eventually developed signs similar to those of the colony from which the original isolate was made. In Japan, Nasu and co-workers previously reported that *Zygophiala jamaicensis* caused flyspeck on persimmon, and Zhang et al. documented that *D. mali* was one species in the sooty blotch and flyspeck complex on apple fruit in China. This is the first report that *D. mali* is a causal agent of flyspeck on persimmon.

Interactive effect of arbuscular mycorrhizal fungi, soybean cyst nematode, and soil pH on iron-deficiency chlorosis and growth of soybean
M. Sun (1), S. CHEN (1), J. E. Kurle (1), J. Zhou (1)
(1) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S154

Soybean cyst nematode (SCN) and iron-deficiency chlorosis (IDC) limit soybean yield. Arbuscular mycorrhizal fungi (MF) have beneficial effects on plant growth. The interactive effects of SCN, MF, and soil pH on IDC and growth of soybean were examined in a greenhouse experiment. The experiment was a RCB design with three factors: 1) SCN population densities (0, 500, and 10,000 eggs/100 cm^3 soil), 2) MF treatments (MF and non-MF), and 3) soil pH levels (pH 5.6, 6.9, and 8). Leaf chlorophyll content (LCC) was determined at 5, 6, 7, 8, and 9 weeks after planting. Shoot dry weight was determined 65 days after planting. The interaction of SCN, MF, and pH significantly affected LCC. SCN resulted in greater reduction of LCC in high than in low pH soil, and the effect was greater at later sample dates in high pH soil. MF increased LCC in low pH soil regardless of SCN population density and in high pH soil without SCN. MF reduced LCC if both pH and SCN population density were high. Symptoms of IDC were observed only in pH6.9 and pH8 soil at 10,000 eggs/100 cm^3 soil. MF increased shoot weight at pH6.9 and pH8 but not at pH5.6. SCN reduced shoot weight more at pH6.9 and pH8 than at pH5.6. When MF was present, shoot weight was greatest at pH6.9 with no SCN, and at pH5.6 if SCN population density was high. The study demonstrates that SCN causes greater damage to soybean when interacting with high pH, and that MF had beneficial effect on soybean growth regardless of SCN infection in high pH soil.

Resistance screening of *Festuca arundinacea* to both *Rhizoctonia solani* and *Rhizoctonia zeae* using digital image analysis
V. R. SYKES (2), B. J. Horvath (2), S. E. Warnke (1)
(1) USDA; (2) Virginia Tech
Phytopathology 98:S154

A major disease affecting tall fescue is brown patch, caused by the fungus *Rhizoctonia solani*. A related pathogen, *Rhizoctonia zeae*, presents similar symptoms, but is not usually included in the screening process when developing brown patch resistant cultivars. This exclusion, along with the subjectivity of visual disease severity evaluation methods, may contribute to the variability in brown patch resistance observed among available cultivars. The objective of this study was to use digital image analysis to perform an initial screen of tall fescue plant introductions (PIs) for resistance to both *R. solani* and *R. zeae*. PIs included germplasm obtained from the USDA germplasm database and three commercial cultivars with varying brown patch resistance. PIs were screened for resistance to each pathogen separately. Plants were inoculated with either *R. solani* or *R. zeae*, and maintained in a closed chamber for 12 days, at a temperature ideal for pathogen development. Disease severity of each PI was evaluated using a whole pot digital image analysis method and compared using ANOVA. Results from the initial screen to *R. solani* showed percent disease severity ranging from 0.3% (uninoculated control) to 49%. Disease severity on the three commercial cultivars was the lowest of the plants evaluated, showing no significant difference (alpha = 0.05) from the uninoculated control. Disease severity on three of the nine PIs was not significantly different from the uninoculated control, and disease severity on six of the nine PIs was not significantly different from the commercial cultivars. The *R. solani* screen will be repeated and ongoing work is being done to evaluate the PI's resistance to *R. zeae*.

Differential gene expression during sclerotium formation and development in the southern blight pathogen *Sclerotium rolfsii*
J. E. TAKACH (1), S. E. Gold (1)
(1) University of Georgia, Athens, GA
Phytopathology 98:S154

Sclerotium rolfsii, the causal agent of southern blight or white mold, is a basidiomycete plant pathogen with a host range of over 400 species that includes economically important crops such as tomato, cotton, and peanuts. *S. rolfsii* is a soilborne pathogen that survives and is spread from season to season as small resistant sclerotia. The mustard seed-sized sclerotia germinate midseason under wet or humid conditions to grow as white fluffy mycelia on regions of the plant nearest the soil line. The mycelium typically grows until it exhausts the nutrient supply, then produces sclerotia. Thus, sclerotia are critical for the persistence of disease potential. Although much is known about the environmental conditions required to stimulate sclerotium production, little is known about the molecular mechanisms that are crucial to this process. To this end, we created an EST library of sequences differentially expressed during sclerotium formation and development. Roughly 200 of the 2,000 library clones were identified as containing sequences up- or down-regulated during sclerotium development, which correspond to 20 fungal protein-encoding genes present in the National Center for Biotechnology Information (NCBI) database. Differential expression of a subset of the differential genes has been confirmed by northern blot analysis. The identification of genes and pathways that trigger sclerotium formation will provide new targets to disrupt the life cycle of *S. rolfsii*, stopping or slowing the season-to-season spread of this pernicious pathogen.

Novel *Pseudomonas* strains antagonistic to *Rhizoctonia solani*, isolated from subterranean seeds of *Amphicarpa bracteata*
J. TAMBONG (1), R. Xu (1), T. A. Ngwa (1), E. Bromfield (1)
(1) Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, 960 Carling Avenue, Ottawa, Ontario Canada K1A
Phytopathology 98:S154

Rhizoctonia solani is a fungal pathogen that causes economic losses in soybean [Glycine max (L.) Merril]. No effective fungicides are available against *Rhizoctonia* diseases, although chlorothalonil and some other chemicals are sometimes recommended. The use of chemicals for disease management is a growing concern to public health and environment. Biocontrol using antagonistic bacteria has been considered as an alternative strategy. The soybean-related wild legume plant, *Amphicarpa bracteata* (hog peanut), is indigenous to North America. Subterranean seeds of this plant were collected from a natural habitat that had not been subjected to agriculture. Therefore, the seeds were potentially predisposed to harbour unique bacterial diversity that could be exploited as biopesticides. Sixty bacterial isolates obtained from subterranean seeds of *A. bracteata* were grouped into 4 major phylogenetic clusters (*Pseudomonas* spp., *Stenotrophomonas* spp., *Agrobacterium* spp. and *Flavobacterium* spp.) by DNA sequencing of the 16S rRNA, the *gacA* (for global *a*ntibiotic and cyanide control) gene and the 16S rRNA-23S rRNA internal transcribed spacer (ITS1). ITS1 exhibited significant sequence variability among different operons within a single genome. Preliminary *in vitro* evaluation of recovered fluorescent pseudomonands against *R. solani* identified 10 isolates with potential antagonistic activity. In polymerase chain reaction (PCR) tests, genes encoding phenazine-1-carboxylic acid, biosurfactants and pyrrolnitrin were detected in reference strains as previously reported but not in our selected *Pseudomonas* strains, indicating that other antifungal metabolites might be involved in their biocontrol activity. These novel *Pseudomonas* strains are being further evaluated for effective *in vivo* control of *R. solani* on soybean.

Analysis of Cyclophilin involvement in CYDV-RPV transmission by *Schizaphis graminum*
C. TAMBORINDEGUY (1), S. Gray (2)
(1) Cornell University, Ithaca, NY, USA; (2) USDA-ARS, Cornell University, Ithaca, NY, USA
Phytopathology 98:S154

Luteoviruses are transmitted by aphids in a circulative, nonpropogative manner requiring the virus to be actively transported across cell membranes and through cell cytoplasm. Using proteomic analyses we have identified two forms of Cyclophilin in a F2 *Schizaphis graminum* population segregating for the ability to transmit *Cereal yellow dwarf virus* (CYDV)-RPV. Cyclophilin is a peptidyl-prolyl cis-trans-isomerase and has been involved in several functions including macromolecular transport. The cyclophilin gene sequence was determined for several *S. graminum* genotypes that differ in vector competency and also differing in which barrier (hindgut or salivary gland) is operating in the nonvector genotypes. A single point mutation changing a C to G is correlated with genotypes that are unable to transmit virus. The cyclophilin that predominates in the vector genotypes is able to bind to purified virus particles in co-immunoprecipitation assays. Continuing efforts are focused on functional assays to directly determine the involvement of cyclophilin in CYDV movement in the aphid.

Quantification of *Fusarium virguliforme* in soybean roots of partially resistant and susceptible genotypes using quantitative polymerase chain reaction
E. TANG (2), G. L. Hartman (1)
(1) USDA-ARS, Urbana, IL, USA; (2) University of Illinois, Urbana-Champaign, IL, USA
Phytopathology 98:S154

Soybean sudden death syndrome, caused by *Fusarium virguliforme* (syn. *Fusarium solani* f. sp. *glycines*), was first reported in 1971, in Arkansas. Since then, the fungus has spread to the northern United States, causing significant soybean yield losses. Soybean resistance to *F. virguliforme* is considered quantitative, marked by a reduction in foliar symptoms. Few studies have identified any root resistance in soybean. In this study, we developed a method to measure fungal DNA concentrations in inoculated roots and evaluated it using five soybean entries: P3981 (very susceptible), Douglas (susceptible), Forrest (intermediate), Cordell (resistant), and PI567374 (very resistant). Radicles from germinating seeds were inoculated using a mycelial plug of F. virguliforme. Four DAI, fungal DNA was extracted from the

radicles and measured by quantitative PCR. The amount of *F. virguliforme* DNA found in Pioneer 3981 and PI 567374 was 1.46×10^{-1} ng/mg and 1.96×10^{-3} ng/mg of host tissue, respectively. Based on the student t-test he mean concentration of fungal DNA from Pioneer 3981 was significantly higher than that from Forrest, Cordell, and PI 567374, while PI 567374 had significantly lower fungal DNA concentrations than Pioneer 3981, Douglas, and Forrest. These results indicate that quantitative PCR has potential to quantify fungal colonization in roots and may be a useful tool for screening soybean accessions for root resistance to *F. virguliforme*.

Development of real-time quantitative assay for rapid detection of *Gliocladium roseum* 67-1, an effective biocontrol agent, in soil

M. Tao (1), S. LI (1), Y. Zhang (1)
(1) Soilborne Diseases Laboratory, Inst. of Plant Protection, Chinese Acad. of Agr. Sci., Beijing 100081, China
Phytopathology 98:S155

Gliocladium roseum 67-1 selected by the authors has been demonstrated to be a highly effective biocontrol agent of the diseases caused by *Sclerotinia sclerotiorum* and *Rhizocotonia solani*. A rapid and accurate quantification of the agent is urgently needed for understanding its interaction with disease pathogens in soil and improving its effectiveness for disease management. Primers and probes for quantification in soil by real-time PCR were designed from ITS regions of the fungi belonging to several taxon. Specificity tests of the primers and probes using genomic DNA indicated that they could specifically amplify ITS rDNA of the biocontrol agent. Standard curve developed by using the plasmid containing the ITS rDNA of the agent gave rise to 0.9989 as linear correlation coefficient between DNA concentrations and Ct values, and 94.9% as amplification efficiency. DNA at 10^{-9} g/ml could be detected. Quantification of the agent added in natural soil by amplifying soil DNA obtained with Mobio UltraClean soil DNA kit showed that Ct value was highly correlated with population density of the fungus (coefficient 0.984). Primary tests on the interaction between *R. solani* AG1 and the biocontrol agent indicated that population of the pathogen could be suppressed by 75% after co-incubation in natural soil for 2 weeks under 20°C and 15% water content although population of the isolate 67-1 declined a lot at the same time.

Molecular identification of viruses that infect *Panax notoginseng* in China

Z. TAO (1), Z. Yan (1), L. Song (1), H. Li (1), Z. Fan (1)
(1) Dept. Plant Pathology, China Agricultural University, Beijing, China
Phytopathology 98:S155

Sanqi [*Panax notoginseng* (Burk) F. H. Chen, PN] is a famous Chinese traditional medicinal herb which grows in Yunnan and Guangxi provinces specifically. Since 1990's, viruses infection caused increasing destructive disease in Sanqi cultivated region and the infected plants showed severe mosaic and/or crimple symptom. In this study, samples showing virus disease symptom was collected from Sanqi plantations. Seventeen indicator plants from 5 families (Chenopodiaceae, Cucuribaceae, Legminosae, Solanaceae and Umbelliferae) were mechanically inoculated with the leaf crude sap of diseased *P. notoginseng*, and all plants showed no symptom in 40 days. Filamentary and spherical particles were found in the leaf sap by Electron microscopy. ELISA revealed the viruses in *P. notoginseng* were not serologically related to all the 29 antisera of 14 genuses stored in our lab. Double strand RNA (dsRNA) was isolated for construction of cDNA library, from which viruses genome segments was cloned randomly. BLAST results showed that there was one potyvirus. Using the potyvirus degenerate primers, the sequence covering regions that coding coat protein (CP) HC-P3 and CI were obtained. Sequence comparison of the amino acids of CP showed that this virus was most closely related to *Carrot virus Y* (CarVY), *Apium virus Y* (ApVY) and *Celery mosaic virus* (CeMV) with percent identities of 75%. Phylogenetic analyses of the sequences indicated that this virus isolate is a distinct potyvirus.

Assessment of fruit resistance to anthracnose in mango cultivars in south Florida

T. L. TARNOWSKI (1), R. C. Ploetz (1)
(1) University of Florida, Homestead, FL, USA
Phytopathology 98:S155

Anthracnose, caused by *Colletotrichum gloeosporioides*, is the most serious disease of mango in humid subtropical and tropical climates. While anthracnose occurs on most plant parts, it causes the greatest economic losses on harvested fruit. There is no total resistance to anthracnose in mango, and previous reports on the resistance of fruit from different cultivars have been almost entirely anecdotal. Objective information on anthracnose resistance among different mango cultivars would be useful for producers and would assist the improvement of this important fruit crop. In 2006, fruits of nine cultivars were rated for anthracnose severity, and in 2007 those from 20 cultivars were rated. Twenty fruits from each cultivar were harvested at maturity, based on fruit color, hardness, ease of release from the tree, and scant sap flow from the pedicel. Disease severity (0.0-1.0) was rated every 3–4 days for 2 weeks with a pictorial scale published by Corkidi et al. (2006). Based on disease levels (y-values) and rates of disease increase (r), the most resistant cultivars were 'Zebda', 'Van Dyke', 'Florigon', and '13-1' (with $y_0<0.01$ and $y_{max}<0.30$), and the most susceptible were 'Sensation', 'Lippens', 'Bullock's Heart', and 'Brooks' (with $y_0>0.04$ and $y_{max}>0.6$). A third year of data will be collected in 2008.

Mango anthracnose in south Florida: Assessing the respective roles of *Colletotrichum gloeosporioides* and *C. acutatum*

T. L. TARNOWSKI (1), R. C. Ploetz (1)
(1) University of Florida, Homestead, FL, USA
Phytopathology 98:S155

Anthracnose is the most important disease of mango. *Colletotrichum gloeosporioides* is indicted most often as the causal agent, but *C. acutatum* has been reported occasionally. To assess the respective roles of each species in south Florida, isolates of *Colletotrichum* were recovered from hierarchically sampled symptomatic leaves and fruit in 2007, and inflorescences in 2008. Isolates were speciated based on conidia and colony morphology, and with PCR using species-specific primers. Only *C. gloeosporioides* was recovered from fruit lesions, but *C. acutatum* was recovered from over 90% of the lesions on new leaves. And *C. gloeosporioides* was recovered from 75% of blighted inflorescences, but *C. acutatum* was recovered from only 13%. In 2008, 3 isolates of each species were used to inoculate healthy panicles in the field, and disease severity was measured six times over 4 weeks. Two of the *C. gloeosporioides* isolates were highly pathogenic (>50% severity at 28 days), but the remaining *C. gloeosporioides* isolate and the *C. acutatum* isolates were weakly or not pathogenic. In the future, we will determine: i) leaf symptoms that are caused by each species, ii) whether the species display the same tissue preferences in other south Florida groves, and iii) the relative pathogenicity of the species on leaves and fruits of mango. This research should increase our understanding of the etiology and epidemiology of anthracnose on mango and may influence its management in the future.

A multilocus phylogeny of the biotypes of *Moniliophthora perniciosa*, cause of witches' broom on cacao

T. L. TARNOWSKI (1), R. C. Ploetz (1)
(1) University of Florida, Homestead, FL, USA
Phytopathology 98:S155

Moniliophthora (formerly *Crinipellis*) *perniciosa* causes witches' broom, one of the most serious diseases of cacao, *Theobroma cacao*. Four biotypes are recognized based on host preference: the C-biotype from *Herrania* and *Theobroma* spp., the S-biotype from solanaceous weeds, the H-biotype from *Heteroptery acutifolia* (designated *Crinipellis brasiliensis* by Arruda et al., 2005), and the L-biotype from liana vines. ITS and IGS sequence phylogenies have provided some, although less than compelling, evidence that the biotypes correspond to distinct phylogenetic lineages. In the present work, phylogenetic relationships among the biotypes were examined further with sequences of mtSSU, ITS, IGS, Ef1alpha, and RPB1 loci. Maximum parsimony analysis was used to construct phylogenies for each locus individually, followed by both maximum parsimony and maximum likelihood analyses to construct phylogenetic trees using a supermatrix containing all loci. Single locus phylogenies of ITS, IGS, and RPB1 distinguished the C and S biotypes, and an H/L biotype clade with moderate to high bootstrap support. Supermatrix analyses resulted in similar trees, but with lower bootstrap support for separating the S and H/L clades from the C clade. Molecular systematics of *M. perniciosa*, as well as ongoing morphology and pathogenicity studies, will further clarify the evolutionary history of this important pathogen and help assess the threat other biotypes may pose to cacao.

Enzymatic response of cotton plants to the pathogen, *Verticillium dahliae*

J. J. TASHPULATOV (1), T. G. Gulyamova (1), D. M. Ruzieva (1), A. M. Kerbalaeva (1), S. M. Nasmetova (1), R. S. Sattarova (1), S. M. Khodjibaeva (1), R. Stipanovic (2)
(1) Institute of Microbiology, 7B, A.Kadyri street, Tashkent, Uzbekistan, 100128; (2) USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX, USA
Phytopathology 98:S155

Pathogen infection initiates a set of local and systemic responses in plants. These responses include local oxidative burst, which may lead to death of infected cells, and changes of cell walls composition in neighbouring tissues, and *de novo* synthesis of antimicrobial compounds (phytoalexins) and PR-proteins. The enzymatic response of resistant and susceptible cotton varieties to the pathogenic fungus *V. dahliae* was studied. The enzymatic activity was measured in cell free extracts of roots, stalks and leaves of 30-days old plants to determine efficiency of intercellular signal distribution after pathogen

treatment. Comparative analysis of the distribution of specific enzymatic activities in tissues of resistant and susceptible varieties before and after infection, revealed the primary localization of activity (except chitinase and peroxidase) was in roots. Although qualitative changes of enzymatic activities in resistant and susceptible cotton varieties were similar, there were significant quantitative differences. Specifically, all of the studied enzymes except amylase were higher in the resistant variety. The highest enzymatic activity in both varieties was located in roots, except peroxidase; the activity of the latter was highest in leaves. The increase of enzymatic activity in response to pathogen action testifies to a defensive reaction in both varieties. Expressed activity in resistant and susceptible plants was highest in roots – the place of direct contact between the plant and pathogen. Thus, the data suggests that the systemic increase of chitinase, peroxidase, glucanase and xylanase activities may be considered part of cotton's defensive reaction in response to the pathogenic fungus *V. dahliae*.

Delineation of fungal species within the genus *Pseudocercosporella* in the sooty blotch and flyspeck complex

N. TATALOVIC (1), J. Batzer (1), M. Diaz (1), M. L. Gleason (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 98:S156

The sooty blotch and flyspeck (SBFS) fungal complex causes major losses to apple growers in humid regions of the world. From a collection of 137 isolates in the genus *Pseudocercosporella* from the eastern and midwestern U.S., parsimony analysis of the Internal Transcriber Spacer region of rDNA delineated 8 putative species. Growth and morphology of representative isolates of each putative species were compared. Optimum temperature for mycelial growth on malt extract agar varied from 20 to 25°C, and growth rate differed among putative species. On potato dextrose agar, one or more features of colony morphology (conidial shape, size and color; presence of soluble pigments in agar; and colony shape, texture, color, and height) were distinct among the following groups: putative species RH1.4; RH2.1 and RH2.3; RH2.3 and RH 2.4; RH1.4; and RH5.1. The distinctions noted in this study will complement sequence information in clarifying the taxonomic status of these fungi and enabling assignment of Latin binomials to the putative species.

Development of a rapid detection method for *Erwinia amylovora* by loop-mediated isothermal amplification (LAMP)

T. N. TEMPLE (1), V. O. Stockwell (1), K. B. Johnson (1)
(1) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902
Phytopathology 98:S156

Detection of plasmid (pEA29) and chromosomal DNA of *Erwinia amylovora* by a loop-mediated isothermal amplification protocol (LAMP) was evaluated in laboratory assays. LAMP amplifies target DNA rapidly (1 hour), isothermally (65°C), and with high-specificity based on four primers designed to recognize six independent sequences of target DNA. A positive reaction results in a cloudy white precipitate of magnesium pyrophosphate in a PCR tube. With whole cell suspensions, our LAMP protocol had a detection limit of 5 to 25 colony forming units, which was similar to the sensitivity of nested PCR. The LAMP primers did not react with suspensions of *Pantoea agglomerans*, *Pseudomonas fluorescens*, and *P. syringae*. In experiments with apple, 0, 10, 100, or 1000 flowers collected from orchards were added to 5 liters of distilled water containing 0, 500, or 5000 CFU per ml of *E. amylovora*. Flower washes were sampled directly, after filtration, or after centrifugation prior to a LAMP reaction. The LAMP reaction detected *E. amylovora* in 96% of samples with the pathogen and had no reaction with non-pathogen amended controls. The number of flowers in the suspension had no effect on LAMP results. Extracting DNA improved the incidence of detection in all assays. A similar experiment with pear flowers resulted in 96 and 8% positive detection with LAMP for suspensions with and without the pathogen, respectively. Indigenous bacteria were isolated from flower suspensions at densities of 1.2×10^4 CFU/ml for apple and 3.9×10^4 CFU per ml for pear. When coupled with disease forecasting models, the ability to rapidly detect epiphytic populations of *E. amylovora* with LAMP could improve fire management.

Wood modifications by brown rot fungi may offer competitive advantage for their own cellulases

J. P. TEWALT (1), J. S. Schilling (1)
(1) University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S156

Despite their remarkable ability to degrade biomass it is still unclear how brown rot wood degrading fungi achieve such high degradation levels without an active cellobiohydrolase (CBH) to cleave crystalline cellulose. We propose that modifications early in the decay process function to selectively benefit the abbreviated cellulase system of brown rot fungi. Preliminary results were conducted with pretreated wood blocks by the brown-rot fungi *Gloeophyllum trabeum*, and *Fomitopsis pinicola* for time periods of 0, 1, 2, 4, and 8 weeks. The pretreated blocks were then treated with the respective brown rot extract, *Trichoderma reesei* crude extract, and commercial *Trichoderma reesei* (Celluclast). Optimization of harvesting to achieve high cellulase activity was conducted prior to treatment. The diverse cellulase activities were controlled by using endo-cellulase as the basis for saccharification addition. Saccharification samples were then analyzed at 2 and 5 days using high performance liquid chromatography (HPLC) to measure glucose concentrations. In preliminary results *Gloeophyllum trabeum* cellulases received an added benefit over the *Trichoderma reesei* cellulases after 4 weeks of pretreatment. The increased efficiency in pre-treated samples could be related to beneficial modifications that are specific to the *Gloeophyllum trabeum* cellulase system. Further identification of the timing and mechanisms that illicit modifications may help solve the problems associated with recalcitrant lignocellulose in biofuels, or aid in the development of targeted biocides.

Evaluation of the genetic structure of *Xylella fastidiosa* populations collected from almond orchards in California

S. THAMMIRAJU (3), K. Daane (2), R. Groves (4), H. Lin (1), M. Sisterson (1)
(1) USDA-ARS, Parlier, CA, USA; (2) University of California, Berkeley, CA, USA; (3) University of California, Berkeley/USDA, CA, USA; (4) University of Wisconsin, Madison, WI, USA
Phytopathology 98:S156

Xylella fastidiosa causes many economically important crop diseases including Pierce's disease of grape and almond leaf scorch disease. A more detailed understanding of the genetic structure of *X. fastidiosa* populations is likely to aid in the development of novel management strategies. Current evidence indicates that strains of *X. fastidiosa* are often clustered within groups or pathotypes based upon host association. Thus, we used simple sequence repeat (SSR) markers to examine the effects of cultivar and geographic location on *X. fastidiosa* populations collected from almond. Analysis of samples collected from almond orchards in different counties indicated that *X. fastidiosa* populations collected from different cultivars within the same orchard were more dissimilar than *X. fastidiosa* populations collected from the same cultivar from geographically separated orchards. We are currently screening samples from an additional two counties to more fully assess the effects of geographic distance on *X. fastidiosa* population structure.

Grafting – A tool for managing root-knot nematodes in watermelon?

J. A. THIES (2), J. J. Ariss (2), C. S. Kousik (2), R. L. Hassell (1)
(1) Clemson University, Coastal Research and Education Center, Charleston, SC, USA; (2) U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC, USA
Phytopathology 98:S156

Wild watermelon (*Citrullus lanatus* var. *citroides*) germplasm, bottlegourd (*Lagenaria siceraria*), hybrid squash (*Cucurbita moschata* × *C. maxima*), and commercial wild watermelon (*C. lanatus*) were evaluated as rootstocks for cultivated watermelon (*C. lanatus* var. *lanatus*) in a field infested with the southern root-knot nematode (RKN, *Meloidogyne incognita*) in Charleston, SC. 'Fiesta', a diploid watermelon, was grafted on the rootstocks and transplanted to the field on 30 July 2007. On 8 Oct 2007, roots were lifted and evaluated for percentage of roots galled by RKN. Root galling of bottlegourd and squash hybrid rootstocks was severe (80 to 96% and 98%, respectively), and galling was moderately severe (40%) for non-grafted 'Fiesta' watermelon. Rootstocks of wild watermelon germplasm derived from *C. lanatus* var. *citroides* had 11 to 34% galling of root systems and the commercial wild watermelon rootstock had 24% galling. Four of five *C. lanatus* var. *citroides* germplasm lines and the commercial wild watermelon rootstock had significantly less ($P < 0.05$) root galling than 'Fiesta', the squash hybrid rootstock, and bottlegourd rootstocks. Germplasm lines derived from *C. lanatus* var. *citroides* may provide useful sources of resistance for development of RKN resistant rootstocks for watermelon.

The status of powdery scab disease on potatoes in Egypt

A. TOHAMY (1)
(1) Plant Pathology Research Institute, Giza,12619, Egypt
Phytopathology 98:S156

Powdery scab of potato caused by *Spongospora subterranean* f. sp. *subterranea* has become an important disease in Egypt during winter season (export season) and summer season (seed production season). The disease was observed on potato tubers harvested from potato growing fields in Behira, Ismailiya and Sharkiya governorates during 2005–06 and 2006–07 seasons. The disease became endemic in potato fields after planting powdery scab-infected potato seeds which led to soil contamination with the powdery scab pathogen. In addition, variations in some edaphic factors such as soil types,

soil pH, temperature and moisture favor the development of the disease. Symptoms of powdery scab on tubers were assessed visually by following the progress of the development of the powdery scab lesions on the infected tubers. The pathogen was identified microscopically according to the morphological features of cystosori (sporeballs) of the pathogen present in the developed lesions on the infected tubers. The effects of edaphic factors, (soil types, moisture, temperature, and pH) on the incidence and severity of powdery scab disease were investigated on potato grown in greenhouse conditions. Both sandy and loamy soils encouraged the development of powdery scab symptoms on potato tubers while clay soil did not. Continuous soil moisture resulted in greater disease severity compared to a fluctuating moisture regime. Disease symptom development was highest in a range of 12–18°C. In addition, the optimum soil pH for the powdery scab disease development ranged between 6 and 7 while at pH 8 the disease symptoms were not noticed. According to the available literature, this is the first record of powdery scab disease in Egypt.

Seasonal activity of leaf spot pathogens of bermudagrass

M. TOMASO-PETERSON (1), J. Young (1)
(1) Mississippi State University, Mississippi State, MS, USA
Phytopathology 98:S157

The purpose of this study was to determine the seasonal activity of the pathogens responsible for inciting lesions on foliar tissue of bermudagrass characteristic of leaf spot. Symptomatic foliar tissue was collected, April through October 2007, from seven bermudagrass cultivars. The tissue included pin-point to well-developed leaf spot lesions and was surface disinfested, plated onto water agar and incubated five days on a laboratory bench top. The tissue was examined for fungal colonization using light microscopy. Fungal colonization was similar for all cultivars therefore the data was pooled. *Bipolaris cynodontis* colonized all tissue pieces collected in April and May, decreasing to 44% in June and averaged less than 6% July through October. *Bipolaris spicifera* colonized plated tissue at low levels (<6%) each month. *Curvularia* spp. were not observed in April, but were observed colonizing plated tissue in May (29%), increasing to 95% in September. *Exserohilum rostratum* was observed colonizing tissue at low levels each month except April. Based on these results, *B. cynodontis*, a causal organism of leaf spot, was the most predominant pathogen observed in the spring while *B. spicifera*, another causal organism of leaf spot, was active at low levels each month. *Curvularia* spp., not documented as leaf spot pathogens, were the most predominant fungi colonizing symptomatic foliar tissue June through October of 2007. *Bipolaris* spp. inciting leaf spot of bermudagrass were observed colonizing symptomatic tissue each month; however, *Curvularia* spp. appeared the most aggressive colonizers of necrotic tissue. This is consistent with *Curvularia* spp. senectopathic activity.

Tier risk assessments of biopesticides

G. S. TOMIMATSU (1), W. R. Schneider (1)
(1) U.S. Environmental Protection Agency, OPPTS/OPP/BPPD, Washington, DC 20460
Phytopathology 98:S157

Pesticides manufactured from naturally-occuring substances (e.g., pheromones), microorganisms (e.g., *Bacillus thuringiensis*) and genetically-modified organisms are designated as biopesticides. They are distinguished from conventional chemical pesticides by their unique modes of action, low volume use, target species specificity or natural occurrence, and are important components in IPM strategies. Before a pesticide is registered in the U.S., critical science-based risk assessments of pesticides are conducted, which help to provide public confidence. The Office of Pesticide Programs (OPP) in the U.S. Environmental Protection Agency (US EPA) uses the standard tiered risk assessment paradigm of analyzing hazards and exposures to assess pesticidal risks to human health and to the environment. Under a tiered structure, potential risks are determined first from estimates of hazard and exposure under "worst-case" scenarios (Tier I). Subsequent testing (Tiers II, III and IV) may be required to assess adverse effects under more realistic conditions and when lower-tiered studies suggest potentially unacceptable risks. These estimates are developed from a synthesis of test results, intended uses and the open literature to fulfill data requirements addressing the primary disciplines of product analysis and manufacturing, mammalian health and ecological/ environmental effects. The U.S. EPA has over 30 years of regulatory experience in preparing risk assessments for registering biopesticides. In 1984, the U.S. EPA first published pesticide data requirements for all pesticides, subsequently these requirements have been modified periodically to refine the risk assessment and eliminate unnecessary testing for biopesticides. The biopesticide data requirements were recently updated, clarified and revised in December 2007. The poster presentation will provide an overview of a risk assessment, relevant definitions and updated revisions of the current data requirements for the registration of a biopesticide.

Identification of powdery mildews anamorphs (Order Erysiphales) from Puerto Rico

D. Torres (1), L. I. RIVERA-VARGAS (1)
(1) University of Puerto Rico
Phytopathology 98:S157

Worldwide, powdery mildew (Ascomycetes; Order Erysiphales) causes economic losses on a large variety of plants. In Puerto Rico, taxonomic studies related to this order of fungi have been very limited. A major constrain is that their teleomorph is frequently absent, which is an important criteria for their identification. To precisely identify their species, the broad based anamorph identification of powdery mildew as Oidium sp. needs to be resolved, especially those of economic importance. Thus, the basis of this research was to identify powdery mildews using different techniques such as Scanning Electron Microscopy (SEM) of infected tissues and sequencing of the nuclear internal transcribed spacer (ITS) of ribosomal DNA (rDNA) gene. On the basis of SEM examination, the following powdery mildews have been identified: *Erysiphe australiana* in *Lagerstroemia indica* (crabmyrtle); *Erysiphe poligoni* in the weeds, *Desmodium podocarpus* and *Euphorbia heterophyla*; *Golovinomyces cichoracearum* in two weed legumes; and *Leveillula taurica* in onions. Nested PCR of the ITS region of the rDNA produced fragments of 750 to 766 bp. DNA sequencing of these products are in progress. Identification of the powdery mildews will enable us to establish protocols to handle symptomatic introduced plant material and to apply appropriate controls in case of an outbreak. Finally, the data collected will allow for the revision of prior reports of Erysiphales in order to identify the arrival of new species into the island. This effort will be fundamental in establishing guidelines for quarantine or to implement management strategies for invasive species.

Differentiation of *Xylella fastidiosa* subspecies *piercei* isolates from a Texas vineyard into strain groups utilizing simple sequence repeat markers

C. P. TORRES (1), D. N. Appel (1), L. Morano (2)
(1) Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX, USA; (2) University of Houston-Downtown, Department of Natural Science, Houston, TX, USA
Phytopathology 98:S157

Xylella fastidiosa subspecies *piercei* is the causative agent of Pierce's Disease of grape and has caused significant crop stress and loss in vineyards throughout Texas. While multiple techniques are available to identify subspecies of *X. fastidiosa*, only simple sequence repeat markers can be used for the differentiation of isolates within individual subspecies. In this research, we will utilize SSR markers to demonstrate the presence of multiple strains of subsp. piercei within a single vineyard. We will also relate these strains to grape cultivar and vineyard location. Using conventional PCR, 5 SSR markers were used to create banding profiles for 102 isolates collected from 7 grape cultivars planted in 5 blocks throughout a single Texas vineyard. SPSS V13 was used to execute a hierarchical cluster analysis to generate a dendrogram clustering isolates into 3 strain groups ranging in size from 14 to 41 isolates. The smallest and most distantly related cluster contained only 3 cultivar types from 3 blocks. The other clusters contained 6 or 7 cultivar types and represented all blocks. Lastly, 22 pairs of isolates extracted from different parts of the same plant were included. Of these, less than 10% were clustered into separate strains. This study not only shows that there are at least 3 strains of *X. fastidiosa* subsp. *piercei* within this vineyard, but also provides evidence of multiple strains occupying a single plant host. There is no evidence however showing preferential selection of strains for cultivar type or location. This research while demonstrating the diversity of this pathogen has also furthered our knowledge of its epidemiology showing that while multiple strains exist, they do not seem to be distributed in a distinct manner.

Assesment of haplotype diversity at two spot blotch resistance genomic regions among a set of barley resistance sources

C. Torres Puyo (1), J. Albin (1), S. Rodriguez (1), S. Pereyra (2), C. PRITSCH (1)
(1) Biologia Vegetal, Facultad de Agronomía, Universidad de Uruguay; (2) INIA-LE Uruguay
Phytopathology 98:S157

Spot blotch caused by *Cochliobolus sativus* is a major disease for malting barley production in Uruguay. A large number of barley lines are evaluated in field nurseries yearly and those selected are included in pre-breeding schemes. Genomic characterization focused at specific major QTLs for spot blotch resistance could also contribute to identify valuable genotypes. Actually, haplotype diversity at these regions i.e. particular combinations of marker alleles, may unveil non redundant resistance sources. We evaluated haplotype diversity at two major QTLs previously reported for cv. Morex: i) *Rcs-qtl-1H-6-7* (adult resistance, interval ABG500A-ABG452, ca. 10 cM), ii) *Rcs-qtl-7H-2-4* (seedling and adult resistance, interval ABC151A-ABC158, ca. 15 cM).

Sixty barley genetic maps (50 single and ten consensus) were interrogated to select highly informative markers placed within each map intervals. Four SSRs markers were identified: GBM1326 y EBmac0603 at *Rcs-qtl-7H-2-4* and HvALAAT y HVM20 at *Rcs-qtl-1H-6-7*. DNA from twelve barley spot blotch resistance sources (including cv. Morex) were amplified using appropriated SSR primers and migrated by PAGE. The number of alleles detected per marker was three for GBM1326, four for EBmac0603 and four for HVM20. HvALAAT was monomorphic. Haplotypes at *Rcs-qtl-1H-6-7* from three out of eleven genotypes coincided with Morex's. However, at *Rcs-qtl-7H-2-4*, no coincidence with Morex haplotype was observed. Combining haplotypes at the two QTLs, four new haplotypes were detected. Also, no clear association between the expression of seedling and/or adult plant resistance with neither Morex haplotypes was observed. This may indicate that novel and diverse resistance genes may be available. Data mining of barley genetic maps and haplotype diversity analyses at major QTL regions may complement field evaluation and facilitate educated selection of useful resistance sources.

Characterization of HopA1, a *Pseudomonas syringae* type III effector protein

T. Y. TORUÑO (1), M. Guo (1), J. R. Alfano (1)
(1) University of Nebraska-Lincoln
Phytopathology 98:S158

Pseudomonas syringae is a host specific plant bacterial pathogen that requires a type III secretion system (T3SS) to translocate effector proteins into plants for pathogenicity. The type III effector protein HopA1 (formerly HopPsyA) was first characterized in *P. syringae* pv. syringae 61 encoded by a gene located in a pathogenicity island that also encoded the T3SS apparatus. Another strain, *P. syringae* pv. tomato DC3000, also contains a *hopA1* allele but in a different pathogenicity island. In both DC3000 and 61, *hopA1* is preceded by the gene *shcA*, which encodes a type III chaperone for HopA1. When expressed in tobacco (*Nicotiana tabacum* cv. Xanthii), HopA1 elicits a hypersensitive response (HR), a programmed cell death associated with defense and consistent with HopA1 being recognized by a resistance protein inducing effector-triggered immunity. We have determined that the C-terminal portion (98-375 aa) of HopA1 is sufficient for elicitation of HR on tobacco when the DNA is transiently delivered into plant cells using *Agrobacterium*-mediated transformation, suggesting that the C-terminal portion of HopA1 is likely the portion that is recognized. Several *hopA1* alleles from other *P. syringae* strains have been cloned and transiently expressed in tobacco by *Agrobacterium* delivery. We found that all of the alleles tested thus far encode proteins that are capable of triggering an HR in tobacco. Interestingly, HopA1 is lethal when expressed in yeast and its avirulence domain is required for yeast cell death. We are currently determining HopA1's effects on pathogenicity. In addition, we are currently identifying *hopA1* alleles from additional *P. syringae* strains to determine the conservation of their encoded proteins. Our work should determine whether HopA1 has evolved via diversifying or purifying selection and help to elucidate HopA1's roles in host specificity and pathogenicity.

Two plant pathogenic *Pseudomonas* species causing new diseases of spaghetti squash in Quebec

V. TOUSSAINT (1), M. Cadieux (1), M. Ciotola (1), C. E. Morris (2)
(1) Agriculture and Agri-Food Canada, HRDC, St-Jean-sur-Richelieu, Qc, Canada; (2) INRA, UR407, Montfavet, France
Phytopathology 98:S158

In the last years, unusual symptoms were observed on twigs, leaves and fruits of spaghetti squash in southern Quebec. Symptoms were essentially necrosis on leaves and cankers on twigs. On fruit, two predominant symptoms were observed: the first was a green halo on the fruit surface of about 0.5 cm diameter covering a cavity in the fruit flesh, the other symptom was water soaked lesions which merged as the disease progressed and caused the flesh to collapse. In 2007, we recovered more than 200 bacterial isolates from about 150 samples bearing these symptoms. In this bacterial collection, 154 were Gram negative, 89 of which showed a positive HR on tobacco. Among these, 78 were fluorescent on KB. For the fluorescent strains, arginine dihydrolase and esculin hydrolysis as well as ice nucleation activity were determined. From the preliminary results, we found two major *Pseudomonas* profiles. Lesions on leaves and twigs as well as the green halos with cavity were mostly associated to *Pseudomonas syringae* and the water soaked lesions on fruits were mostly associated with *Pseudomonas marginalis*, a soft rot pathogen. For the latter, pectolytic activity was confirmed on potato slices. A few strains of each group were inoculated on plants or fruits and they were able to cause symptoms. This study confirms that this type of water soaked lesions on fruit is caused by *P. marginalis* and not *Fusarium* spp. as sometimes previously stated. Proper disease management of these diseases requires further information on the epidemiology as well as on the resistance of these strains to copper.

Effect of living and straw mulches on yield and disease incidence for spaghetti squash in southern Quebec

V. TOUSSAINT (1), M. Ciotola (1), R. Bacon (1), M. Cadieux (1)
(1) Agriculture and Agri-Food Canada, HRDC, St-Jean-sur-Richelieu, Qc, Canada
Phytopathology 98:S158

Spaghetti squash is subject to several soilborne plant pathogens during field production. In summer 2007, trials were conducted to test living mulches (rye and wheat) and straw has a physical barrier to reduce disease on a commercial and experimental farm, both located in southern Quebec. Living mulches were seeded (May 24th) one week prior to squash (June 1st) and straw was spread to cover the ground at the three leaf stage (June 28th). For both sites, the number of squash and the incidence of diseases were recorded before harvest on a 25 m2 area on each of 4 replicates per treatment. On the commercial farm, the number of squash for all treatments was not significantly different from the conventional crop (control), however, the yield was significantly lower for the rye compared to straw or wheat ($P = 0.05$). The mulch treatment did not have a significant effect on the number of disease free squash ($P = 0.40$). On the experimental farm, treatments had a significant effect on the yield ($P = 0.0009$) as well as on the percentage of disease free squash ($P = 0.0002$). The number of squash was significantly lower with rye and wheat compared to control and straw. Also, the percentage of disease free squash was significantly higher on straw (80%) and lower (33.9%) on rye compared to control or wheat mulch. Results after this first year of trial show that rye seeded in spring reduced yield and did not reduce disease development. None of the treatments had a significant effect on disease development on fruit after one month in storage. In light of these results, further studies on spaghetti squash should privilege the use of inert mulches such as straw.

Identification of amino acids that improve biological control of anthurium blight through inhibition of *Xanthomonas axonopodis* pv. *dieffenbachiae*

P. J. TOVES (1), T. S. Vowell (1), A. M. Alvarez (1)
(1) University of Hawaii, Honolulu, HI, USA
Phytopathology 98:S158

Populations of beneficial bacteria used as biological control agents (BCAs) progressively decrease after the initial foliar application on anthurium plants, reducing their efficacy against anthurium blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Xad). Amino acids representing various biosynthetic pathways were investigated for their potential to enhance BCA survival while reducing Xad growth via feedback inhibition. Valine and isoleucine inhibited growth of Xad *in vitro* when used individually. The inhibition was reversed when these amino acids were used together or when either was used in combination with glutamine or glutamic acid. In greenhouse studies, foliar applications of amino acids together with BCAs reduced the severity of leaf infection by 30 – 40% when compared to the untreated control, and up to 10% when compared to the BCA treatment without amino acids. When applied in the field, disease incidence on anthurium plants treated with amino acids combined with BCAs was reduced by 33% compared to the untreated control, and 22% compared to the BCA treatment without amino acids. The use of amino acids as inhibitors of a bacterial pathogen is a novel approach to enhancing biological control of anthurium blight.

Biological control of peach leaf curl with *Bacillus subtilis*

J. A. TRAQUAIR (2), A. Svircev (1), B. Singh (1)
(1) SCPFRC, AAFC, London ON Canada; (2) Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London ON Canada N5V 4T3
Phytopathology 98:S158

Peach leaf curl is caused by *Taphrina deformans*. This fungus infects peach buds as they break in cool, damp spring weather in most peach-growing areas of Canada and USA. Red, gall-like blisters containing a dense palisade of asci below the leaf epidermis, are produced on wrinkled and curled, developing leaves. Resulting defoliation can reduce fruit set and yield. Fungicides such as dithiocarbamates (Ferbam) applied to dormant twigs and buds prevent infection by overwintered ascospores and blastoconidia. Serenade MAX (*Bacillus subtilis*, strain QST713) developed by AgraQuest, Inc., was evaluated as an alternative to sprays of peach trees with chemical fungicide. Using *in vitro* bioassays, Serenade MAX inhibited the growth of *T. deformans* by secretion of a diffusible compound(s). In the orchard, peach leaf curl was suppressed by one and two applications of Serenade Max at 6.6 kg/ha using Biotune as a surfactant, compared to the Ferbam standard. Total yield of peaches was increased by use of the Serenade MAX biocontrol compared to the chemical fungicide standard. Serenade Max will be a useful tool for organic peach growers in Canada.

Fungicide sensitivity in North Carolina populations of *Colletotrichum cereale* and molecular characterization of benzimidazole- and QoI-insensitive strains

L. P. TREDWAY (1), M. D. Soika (1), M. L. Bunting (1)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC
Phytopathology 98:S159

Isolates of *Colletotrichum cereale* were obtained from creeping bentgrass and annual bluegrass putting greens exhibiting symptoms of anthracnose basal rot or foliar blight. Seventy-one isolates were obtained from 6 locations in NC and 1 location each in VA and TN. Sensitivity to thiophanate-methyl, azoxystrobin, and propiconazole was determined *in vitro*. All 71 isolates grew uninhibited on media containing 10 µg/ml thiophanate-methyl. Sequence analysis of the TUB1 and TUB2 genes from selected isolates revealed E198K and E198A amino acid substitutions in TUB2. One isolate was sensitive to azoxystrobin, 9 were moderately sensitive, and the remaining isolates were insensitive to azoxystrobin. Sequence analysis of the CYTB gene in selected isolates revealed an F129L amino acid substitution in moderately sensitive isolates and a G143A amino acid substitution in insensitive isolates. All F129L isolates were obtained from a single location in Western NC, where application of QoI fungicides provided approximately 50% control of anthracnose in replicated trials. EC_{50} values for propiconazole ranged from 0.27 to 1.33 µg/ml. Resistance to the benzimidazole and QoI fungicides has become widespread in *C. cereale* populations in NC and surrounding states, and some locations are also likely to observe reduced efficacy from DMI fungicides. Populations dominated by isolates with F129L mutations in CYTB may be partially suppressed by QoI fungicide applications.

***Phakopsora pachyrhizi* gene expression during infection in soybean**

A. TREMBLAY (1), H. S. Beard (1), S. Li (2), B. E. Scheffler (2), B. F. Matthews (1)
(1) USDA-ARS, Beltsville, MD, USA; (2) USDA-ARS, Stoneville, MS, USA
Phytopathology 98:S159

Soybean is one of the top five agricultural products in the United States with a value exceeding $19.7 billion in 2006. Protection of soybean from present and new exotic pathogens is very important for soybean production. Asian Soybean Rust (ASR), *Phakopsora pachyrhizi*, is an exotic pathogen first observed in the United States in 2004 in Louisiana. Since then, ASR has gradually moved north into the U.S. major production areas. This pathogen causes yield losses due to premature defoliation, fewer seeds per pod and decreased number of filled pods per plant. In this perspective, we will identify genes from Asian Soybean Rust that might be involved in the early infection steps of either spore germination or appressorium formation and also soybean genes that may be useful to control ASR. Thus, we constructed and analyzed cDNA libraries to identify candidate genes. One library was constructed from leaves heavily infected with ASR (7 to 10 days after infection). The second library was constructed from RNA isolated from uredinium formed by the ASR on the under side of leaves. Uredinia were isolated by laser capture microdissection. A portion of each library was sequenced, contigs were formed, and blast searches were conducted to determine the identity of the genes. In the future, target pathogen genes will be studied to determine if they can be used to prevent disease development. Also, we will examine the selected candidate genes to determine if they can be used to control ASR in soybean.

Accelerated degradation of metam-sodium in soil: Occurrence and possible mechanism

S. Triky-Dotan (1), M. Austerweil (1), D. Mintz (1), Y. Katan (2), A. GAMLIEL (1)
(1) ARO Vocani Center, Bet Dagan Israel; (2) Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel
Phytopathology 98:S159

Accelerated degradation (AD) of soil fumigants can result in insufficient pest control. We have documented AD and reduced effectiveness of Methyl-isothiocyanate (MITC), the active ingredient of Metam-sodium in soil, resulting from repeated applications under field conditions. AD of MITC may result from enrichment in the population of microbial degraders in the soils, from increased enzymatic activity of the degraders, from the transfer of extrachromosal elements from the degraders to the other components of the soil microbial community, or from a combination of these factors. Revealing the microbial mechanism underlying this AD may provide tools for managing it. The objective of the present study was to identify and characterize the microorganisms responsible for the occurrence of accelerated degradation of MITC in soil. Under controlled conditions, we were able to induce accelerated degradation of MITC in six different agricultural soils, by inoculating natural (nonhistory) soil with 10% of MS history soil (three repeated MS applications). Disinfestation of a history soil by steam or solarization eliminated the AD phenomenon. In contrast, fumigation with methyl bromide, iodomethane, or formalin did not reduce AD in history soil. We developed a soil extraction method in order to increase the density of the degrading organisms. Inoculation of nonhistory soil with such extract from a history soil resulted in AD in the soil, indicating the presence of degraders in the extract. Liquid culture of the soil extract from history soil had a high potency for rapidly degrading MITC. Heating the soil extract from a history soil to 60°C for two hours eliminated the accelerated degradation. Initial results indicate that the DNA profiles in history and nonhistory soils after MS application are different. We isolated culturable gram negative bacteria which rapidly degrade MITC in liquid culture as well as in soil.

Transmission of *Grapevine leafroll-associated virus* 3 by the vine mealybug (*Planococcus ficus*)

C. TSAI (1), J. Chau (1), L. Fernandez (1), D. Bosco (2), K. M. Daane (1), R. P. Rodrigo (1)
(1) University of California, Berkeley; (2) Universitá degli Studi di Torino
Phytopathology 98:S159

Grapevine leafroll disease is caused by grapevine leafroll-associated viruses (GLRaVs). Within this virus complex, GLRaV-3 is the predominant species in the world. Several GLRaVs have been shown to be transmitted from vine to vine by mealybugs although a detailed characterization of transmission biology is lacking. We studied the characteristics of GLRaV-3 transmission by the vine mealybug. Our results indicate that the vine mealybug transmits GLRaV-3 in a semi-persistent manner. First instars were more efficient vectors than adult mealybugs. Virus transmission occurred with a 1-h acquisition access period (AAP) and peaked with a 24-h AAP. Mealybugs inoculated GLRaV-3 with a 1-h inoculation access period (IAP), and transmission efficiency increased with longer plant access period up to 24 h, after which transmission rate remained constant. GLRaV-3 transmission lacked a latent period in the vector. After an AAP of 24 h, mealybugs lost GLRaV-3 and infectivity 4 days after virus acquisition. In addition, GLRaV-3 was not transovarially transmitted from infected females to their progeny as detected by RT-PCR. In summary, we systematically analyzed transmission parameters of GLRaV-3 by the vine mealybug and showed that transmission of this virus occurs in a semi-persistent manner. This information will be valuable for the development of leafroll disease management practices.

Methyl Iodide and Sulfuryl Fluoride as quarantine treatments for solid wood packing material

K. M. TUBAJIKA (2), A. V. Barak (1)
(1) USDA APHIS CPHST, Cape Cod, MA; (2) USDA APHIS CPHST, Raleigh, NC
Phytopathology 98:S159

The threat of wood-inhabiting fungi to American hardwood forests, lumber industries, and tourism has enormous economic significance, and the aesthetic and dollar values of properties are potentially disastrous. The efficacy of Methyl Iodide (MeI) and Sulfuryl Fluoride (SF) for eradicating wood-inhabiting fungus, *Ceratocystis fagacearum* was assessed in wood blocks of birch, maple, poplar and red pine based on in-vitro experiments. In a series of replicated controlled experiments, wood blocks were inoculated with a 1 g macerated mycelium/spores mixture of *C. fagacearum* and fumigated with 160 and 240 g/m3 of MeI, SF and methyl bromide (Mebr) as control) for 24, 48, and 72 hours. Analysis of variance showed that fumigant types, fumigant concentrations, and exposure time as well as their interactions (C × T) had an effect on *C. fagacearum* recovery on tested wood species. Colonization of birch, maple, red pine, and poplar by *C. fagacearum* was significantly greater in non-fumigated samples than fumigated samples. *C. fagacearum* was greatly inhibited by MeI than SF in all wood species tested. Overall, the C × T products of $\leq$ 4,108 for MeI and $\leq$ 8,755 for SF were not effective in killing the fungus. These results suggest that longer treatment exposure time might achieve the goal of complete eradication of *C. fagacearum* and imply that MeI performed as well as MeBr in killing the fungus in some wood species by exposure time combination. Overall, MeI was most effective in killing the fungus than SF under the conditions of this study with potential implications for quarantine use.

Patterns of multi-virus infections in Florida watermelon

W. W. TURECHEK (1), S. Adkins (1), C. S. Kousik (2), P. A. Stansly (3), P. D. Roberts (3)
(1) USDA-ARS, Horticultural Research Lab, Fort Pierce, FL, USA; (2) USDA-ARS, U.S. Vegetable Lab, Charleston, SC, USA; (3) University of Florida, SWFREC, Immokalee, FL, USA
Phytopathology 98:S159

The whitefly-transmitted viruses Squash vein yellowing virus (SqVYV) and *Cucurbit leaf crumple virus* (CuLCrV) have had serious impact on watermelon production in west-central and southwest Florida in recent years. To determine the distribution of the viruses within plants, we collected 80 entire plants randomly, 20 each on four different dates, from a commercial watermelon field showing symptoms of SqVYV and CuLCrV. Cross sections of

watermelon plants from the crowns through the tips at 2 ft intervals were blotted onto nitrocellulose membranes and nucleic acid hybridization assays were used for identification of SqVYV and CuLCrV. Results showed that SqVYV and CuLCrV were present in the field in approximately 38% and 45% of the 80 plants, respectively, by testing crown tissue. In plants diagnosed with SqVYV, the distribution of SqVYV in vine tissue decreased proportionately with distance from the crown. The probability of detecting SqVYV within the first 2 ft of the growing tip was less than 10%. In contrast, the growing tip was the single best tissue for detecting CuLCrV. The results indicated that SqVYV and CuLCrV are spatially separated in watermelon vines, and this separation may impact how whiteflies acquire and disseminate the viruses.

Evaluating hot-water treatment as means to reduce *Xanthomonas fragariae* in strawberry nursery stock: Field trials

W. W. TURECHEK (1), N. A. Peres (2)
(1) U.S. Horticultural Research Lab, Ft. Pierce, FL, USA; (2) University of Florida, GCREC, Wimauma, FL, USA
Phytopathology 98:S160

Angular leaf spot (ALS), caused by *Xanthomonas fragariae*, is an important disease of strawberry that is transmitted to production fields through infected nursery stock. This creates problems for nurseries who export plants to Europe because of quarantine restrictions and for U.S. growers who expect to receive pathogen-free plants. The southeastern states are particularly vulnerable to ALS due to the likelihood that overhead irrigation will be used for frost protection during the season. In previous studies, we showed that bacterial populations were reduced >99% when exposed to 2 hr at 48°C or 4 hr at 44°C. These same treatments were applied to several different strawberry cultivars in greenhouse trials to determine if the plants could withstand the heat treatment. A few cultivars, notably 'Ventana' and 'Diamante' were severely stunted at 48°C, but faired better at 44°C. In this study, field trials were conducted in Maryland and Florida where several varieties were treated, planted in raised beds, and then evaluated for their ability to withstand heat treatment and rated for the incidence of ALS. The survival rate amongst cultivars was variable, but generally was high for cultivars that showed good tolerance to heat treatment in greenhouse trials. ALS was observed at very low incidence in only a few of the heat-treated plots. However, the incidence of ALS was significantly higher in untreated plots, particularly in Florida, where nearly all control plots developed disease. These results indicate that heat treatment may not be suitable for all cultivars due to their inability to withstand heat treatment, but may be very promising for those that could. We intend to continue with this work and develop a protocol that could be implemented at the production scale.

Studies on *Dulcamara mottle virus* infectious clone and chimeric genomes with *Turnip yellow mosaic virus*

I. E. TZANETAKIS (2), R. R. Martin (3), T. W. Dreher (1)
(1) Dept. of Microbiology, Oregon State University, Corvallis, OR, USA; (2) Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR, USA; (3) USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR, USA
Phytopathology 98:S160

The genus *Tymovirus* consists of viruses with positive sense, single-stranded capped RNA genomes that encode three open reading frames and terminate in a tRNA-like structure. An unpublished report of *Dulcamara mottle virus* (DuMV) 3′ UTR indicated that the virus seemed to terminate in an A-tail, the first such report for a tymovirus. We investigated the genomic 3′ UTR and determined that DuMV has an internal A-track near the terminus of the genome. The complete genome sequence was acquired and phylogenetic analysis showed distant relationships of DuMV with some of the tymoviruses infecting solanaceous hosts, but did not reveal any evolutionary footprint that could give insight into the acquisition or evolution of the 3′ UTR. We developed an infectious clone of DuMV to study the role of the unusual 3′ UTR and made chimeras of *Turnip yellow mosaic virus* and DuMV with their heterologous 3′UTRs. One of the chimeras (TYMVgenome/DuMV 3′UTR) was able to replicate in planta, shedding light in the ability of tymoviral polymerases to recognize different feature in 3′ UTRs and to support replication, indicating the feasibility of the acquisition of the DuMV 3′ UTR during a polymerase-mediated recombination event. These results show that recombination may be a driving force in the evolution of the genus.

Characterization of Verbena virus Y, a new component in the complex causing necrosis in verbena 'Taylortown Red'

I. E. TZANETAKIS (1), S. Cleveland (1), J. Kraus (1), M. L. Putnam (1), R. R. Martin (2)
(1) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA; (2) USDA-ARS Horticultural Crops Research Lab, Corvallis, OR, USA
Phytopathology 98:S160

A 'Taylortown Red' verbena plant from Michigan showed mottling symptoms that progressed to necrosis as leaves matured. The plant was assayed and was found positive for double-stranded RNA, indicative of virus infection, which was cloned and sequenced. Three viruses were identified: *Broad bean wilt virus-1*, Coleus vein necrosis virus and a new potyvirus, the subject of this communication. The complete nucleotide sequence of the potyvirus, referred to as Verbena virus Y (VVY) was determined. The virus polyprotein has the typical *Potyvirus*-associated protein motifs indicating that VVY is a typical member of the genus. Phylogenetic analysis showed that VVY is most closely related to potyviruses that infect solanaceous plants and has greatest similarity to *Potato virus Y* and *Pepper mottle virus* with about 60% nucleotide and amino acid sequence identity. In an effort to determined whether VVY is the causal agent of the necrosis observed in 'Taylor Town' we did mechanical inoculations onto indicator plants to separate the three viruses infecting the plant. None of the 12 species inoculated with 'Taylortown Red' tissue were singly infected with VVY and the only plants that sustained infections belonged to the *Solanaceae*. Trials are under way using aphid vectors to separate the three viruses and reconstruct symptoms leading to the identification of the causal agent(s) of the disease.

Current research results of Puccinia psidii, the guava rust, in Hawaii

J. Y. UCHIDA (1)
(1) University of Hawaii, Honolulu, HI, USA
Phytopathology 98:S160

This invasive rust, first observed on potted ohia, Metrosideros polymorpha, on Oahu has continued to spread to all major islands. It is common on rose apple or Syzygium jambos on all islands in moist locations. Other hosts include paper bark (Melaleuca quinquenervia), Eugenia koolauensis (endangered, endemic), E. reinwardtiana, brush cherry (E. paniculatum), myrtle (Myrtus communis), down rosemyrtle (Rhodomyrtus tomentosa), Java plum (Syzigium cumini), mountain apple (S. malaccense), wax flower (Chamelaucium uncinatum), jaboticaba (Myriciaria cauliflora), Metrosideros tremuloides and a few other hosts. Germination studies have been initiated with urediniospores produced on rose apple plants maintained in the laboratory at 24–25°C next to the window. These plants are grown in the greenhouse, young leaves are painted with urediniospores and the plants are kept moist for 3–4 days. A 24 hr incubation period is sufficient but disease development is faster with longer incubation periods. Pustules are formed in 7 to 10 days, followed by the continuous production of urediniospores. Depending on the leaf age, teliospores may be formed 3–4 weeks after inoculation. The best parameters for urediniospore germination (over 90%) were 0.75% Bacto agar, 20–24°C, 20–24 hrs, and use of fresh spores. Urediniospores formed after 7–12 days are the best. Older spores (a mixture of spores 1 to 20 days old) have over 30% that do not germinate. Fresh spores germinated as well in the light as in darkness.

Genetic and genomic approaches to understand *Phymatotrichopsis* (cotton) root rot of alfalfa

S. UPPALAPATI (3), H. Lee (3), S. M. Marek (1), J. Nakashima (3), M. K. Sledge (2), R. A. Dixon (3), K. S. Mysore (3)
(1) Department of Entomology and Plant Pathology; Oklahoma State University; (2) Lipscomb University; (3) Plant Biology Division, The Samuel Roberts Noble Foundation
Phytopathology 98:S160

Phymatotrichopsis omnivora (Duggar) Hennebert, causes a destructive root rot in cotton, alfalfa (*Medicago sativa*), and many other dicot species. No consistently effective control measures or resistant host germplasm for *Phymatotrichopsis* root rot (PRR) are known. The relative genetic intractability of cotton and alfalfa precludes their use as pathosystem hosts for *P. omnivora* and thus most genomic approaches to study PRR. Therefore, we used the model legume, *M. truncatula* and its available genetic and genomic resources to investigate PRR. In order to screen 250 *M. truncatula* ecotypes for their responses to PRR, a simple, reliable method to infect *M. truncatula* in Houston black clay using *P. omnivora*-infested wheat seeds as inoculum was developed. Using this assay, four ecotypes, PI 505437, PI 239874, W6015 and W6020, with increased tolerance were identified. Using Affymetrix Medicago Genome Array chips, expression profiling of susceptible *M. truncatula* roots infected by *P. omnivora* identified several up-regulated genes, including, the pathogenesis-related Class I and Class IV chitinases, and genes involved in ROS generation and phytohormone (jasmonic acid and ethylene) signaling. Genes involved in isoflavonoid biosynthesis were suppressed in later stages of infection. These transcriptome results, confirmed by real-time quantitative PCR analyses, showed *P. omnivora* apparently evades induced host defenses and may suppress phytochemical defenses at later stages of infection. Taken together, our study identified resistant germplasm and provided new insights into the mechanism of plant susceptibility to PRR.

Use of virus-induced gene silencing and surrogate model *Nicotiana benthamiana* for studying *Pseudomonas syringae* pv. *tomato*-induced chlorosis and cell death

S. UPPALAPATI (3), C. Ryu (2), K. Wang (3), C. L. Bender (1), K. S. Mysore (3)
(1) Department of Entomology and Plant Pathology, Oklahoma State University; (2) Korea Research Institute of Bioscience and Biotechnology; (3) Plant Biology Division, The Samuel Roberts Noble Foundation
Phytopathology 98:S161

Pseudomonas syringae pv. *tomato* (*Pst*) is a serious pathogen of tomato. On tomato, *Pst* causes bacterial speck characterized by dark brown to black lesions of various sizes and shapes on leaves, fruit, and stems. Foliar lesions are generally surrounded by a chlorotic halo due to production of the phytotoxin coronatine (COR). However, the host targets and molecular processes underlying necrotic lesion development and COR-induced chlorosis are poorly understood. In this study we took advantage of a chlorotic phenotype elicited by COR on *N. benthamiana* leaves and high throughput virus-induced silencing as a rapid (reverse and/or fast-forward genetic) screening tool for identification of plant genes involved in coronatine perception and signaling. Fast-forward VIGS screens identified several genes that positively or negatively regulate COR-induced chlorosis and *Pst*-induced disease phenotypes. Furthermore, our reverse-genetic screening identified new members in the COR perception pathway, including SGT1. Pathogenesis assays on the SGT1 gene silenced tomato plants suggests a functional role of SGT1 in necrosis associated with disease development. Taken together, our results indicate that VIGS is a powerful tool for identifying virulence-targets and novel genes involved in disease development.

Evaluation of seed treatments for control of soybean seedling diseases under controlled environmental conditions

K. E. URREA (1), J. C. Rupe (1), C. Rothrock (1)
(1) University of Arkansas
Phytopathology 98:S161

Seedling diseases on soybean frequently reduce seed germination, seedling emergence, stand, vigor, and yield. Environmental conditions shortly after planting play a large role in seedling disease severity. The objective of this research was to evaluate the efficacy of fungicide seed treatments and cultivar resistance for seedling disease control. Soil was collected from a soybean field near Hope, AR, placed in plastic tubs and planted with four cultivars: Hornbeck 4924, Delta Grow 4770, Hutcheson and Archer. The cultivar Archer has been reported to be resistant to several *Pythium* spp. Seed of each cultivar was either not treated or treated with metalaxyl (Allegiance Fl) or mefenoxam+fludioxonil (ApronMaxx RTA). The experiment was conducted at 21°C, and soil water content was maintained above field capacity. There was no significant cultivar by fungicide interaction. After two weeks, Hutcheson and Archer had the highest stands. Differences in stands among the cultivars may indicate differences in disease resistance or seed quality. Over all cultivars, mefenoxam+fludioxonil resulted in the greatest stands followed by metalaxyl.

Survey of plant non-homologous end joining (NHEJ) pathway components for role in *Agrobacterium* T-DNA integration

Z. E. VAGHCHHIPAWALA (1), K. Mysore (1)
(1) The Samuel Roberts Noble Foundation
Phytopathology 98:S161

During *Agrobacterium*-mediated plant transformation, the molecular machinery within *Agrobacterium* which prepares the T-DNA for translocation into the plant cell is quite well characterized. Lately, the mechanism by which T-DNA gets transferred into the plant cell followed by its nuclear import and integration into the host genome has come under rigorous scientific scrutiny. Few plant host proteins for attachment and cytoplasmic trafficking have been identified and characterized, but the involvement and role of plant partners for T-DNA integration into the plant genome remains a grey area. Numerous studies in yeast have identified the involvement of the Non-Homologous End Joining (NHEJ) pathway in T-DNA integration. In plants, Mysore et al. (PNAS 97:948) identified the Histone *H2A* gene to be required for T-DNA integration and Li et al (PNAS 102:19321) established the requirement for the KU80 protein during T-DNA integration. Using the well characterized mammalian NHEJ pathway as a model, our aim is to characterize the components of this pathway and investigate their role in T-DNA integration. To achieve this, we aim to use the well established Virus-Induced Gene Silencing (VIGS) approach in our laboratory as well as *Arabidopsis* mutants for genes involved in NHEJ. The results of a survey of NHEJ components and their role in T-DNA integration will be presented.

Identification of the mating type locus in the *Fusarium oxysporum* species complex associated to chickpea in Mexico

E. VALADEZ-MOCTEZUMA (2), A. Lopez-Lopez (2), L. X. Zelaya-Molina (2), H. V. Silva-Rojas (1)
(1) Colegio de Postgraduados, Texcoco, Edo. de Mexico, Mexico; (2) Universidad Autonoma Chapingo, Texcoco, Edo. de Mexico, Mexico
Phytopathology 98:S161

Chickpea (*Cicer arietinum* L.) is one of the most important export-legume crops in Mexico. Being El Bajio region and Sinaloa State the main producer areas in the country. However, this crop is affected by *Fusarium oxysporum* that cause yellowing and wilt in plant since the firts growth stages. New investigations focused in asexual phytopathogenic ascomycetes have reported the existence of the mating -locus, finding both idiomorphs in different individuals of the same population. In addition, these genes produce functinal proteins that open a new insight into the reproductive biology of this group. By these reasons, the aim of this research was to know the mating type locus present in two populations of *Fusaium oxysporum* species complex associated to yellowing and wilt in Mexico. During 2003–2005, diseased plants of chickpea were recorder from two regions, and seventy seven fungal isolates were obtained. DNA was extracted, and MAT1-1-1, and MAT 1-2-1 genes were amplified, and sequenced. The results showed that in the Bajio region a third of the population present MAT 1-2 and the rest MAT 1-1; by the other hand, all the individuals of Sinaloa population were MAT 1-2. These results give infomration about the presence of both mating type locus in the *Fusarium oxysporum* species complex associated to chickpea in Mexico, and the diferent proportions of each one could help in the advance of the knowledge of the biological reproduction of this group.

***Pythium oligandrum* biocontrol: Influence on fungal populations' dynamics and plant resistance**

J. VALLANCE (1), G. Le Floch (1), F. Déniel (1), P. Rey (2)
(1) Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (EA3882), Plouzané, France; (2) UMR Santé Végétale, ENITAB, Bordeaux, France
Phytopathology 98:S161

In this study, fungal populations and their dynamics were investigated in relation to the introduction of the biocontrol agent *Pythium oligandrum* in the rhizosphere of tomato plants grown in greenhouses. *P. oligandrum* is known to display antagonistic activities against several species of pathogenic fungi, including the ubiquitous tomato root minor pathogens *Pythium* group F, by mechanisms such as mycoparasitism, competition for nutrients and space and production of an elicitor named oligandrin, activating the plant's defences. One limitation to its use is the lack of persistence of the fungus in the rhizosphere. The research was done by inoculating roots of tomato plants grown in hydroponic culture by three selected strains of *P. oligandrum* displaying fairly unique traits. After introduction, these strains were monitored over time to evaluate their persistence and their effect on the microflora. *P. oligandrum* were detected at high rates throughout the 8-months experiment by Real-Time PCR but seems to have a slight influence on the root distribution of the other *Pythium* species, especially on *Pythium* group F. Inter Simple Sequence Repeat (ISSR) analysis performed on *P. oligandrum* isolates collected at the end of the growing season, showed that 90% of the samples belonged to only one of the three selected strains. Single-Strand Conformational Polymorphism (SSCP) and cloning/sequencing investigations point out that the introduction of the antagonistic fungus only has a slight influence on the native fungal community and its dynamics.

Molecular characterization of tomato plant-associated fungal communities after introduction of the antagonistic agent *Pythium oligandrum* in the rhizosphere

J. VALLANCE (1), J. Jany (1), F. Déniel (1), P. Rey (2)
(1) Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (EA3882), Plouzané, France; (2) UMR Santé Végétale, ENITAB, Bordeaux, France
Phytopathology 98:S161

Biocontrol agents are investigated as alternative methods to chemical control in greenhouses to protect plants from pathogenic attacks. However, their impact on the indigenous microflora has not been yet determined. By employing the antagonistic agent *Pythium oligandrum* and tomato plants grown in hydroponic culture as a model, the composition of plant-associated fungal community after the introduction of the biocontrol agent was characterized. The universal primer pair AU2-AU4 was used to amplify the fungal small subunit (18S) rRNA gene from total DNA extracts obtained from roots collected throughout the cultural season from control and *P. oligandrum* inoculated plants. Amplified fungal DNA, containing the V4 region, was cloned, sequenced and searched for chimeric sequences. A selection of 501 clones of 900bp was analysed phylogenetically. Based on sequence similarity, the fungal clone sequences were sorted into different OTUs (Operational Taxonomic Unit). Preliminary analysis allowed no distinction between control and inoculated plants, and dates of sampling. Communities of fungi representing Chytridiomycota, Basidiomycota, Ascomycota and Zygomycota were detected with a predominance of Chytridiomycota. These results contrast

with the small scale culturing analysis carried out to monitor the evolution of four soilless ubiquitous indigenous fungi: *P. oligandrum*, *Trichoderma harzianum*, *Fusarium oxysporum*, and the minor pathogen *Pythium* group F. They were detected at high rates throughout the cultural season but none of them was represented in the results of the sequencing, underlining the difference between cultural and molecular methods in the characterization of microbial communities.

A new virus species causing a disease of Japanese holly fern (*Cyrtomium falcatum*)

R. A. VALVERDE (2), S. Sabanadzovic (1)
(1) Dept. of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, USA; (2) Dept. of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, USA
Phytopathology 98:S162

An RNA virus has been found associated with diseases of Japanese holly fern (*Cyrtomium falcatum*) and leather leaf fern (*Rumohra adiantiformis*). Diseased Japanese holly fern plants show intense yellows, mosaic, necrosis, ring spots, and reduced growth. The virus was successfully graft-transmitted to healthy Japanese holly ferns, with symptoms developing three to five weeks after inoculation. Double-stranded RNA analysis from infected tissues revealed two major dsRNAs considered to be the replicative forms of the viral genome. Viral dsRNAs 1 and 2 were gel purified and used as templates for molecular cloning and sequencing. The genome organization of this novel virus was similar to that of *Raspberry bushy dwarf virus* (RBDV) (genus *Idaeovirus*). Blast searches showed distant relationships with RBDV (RNA-1) and *Groundnut rosette virus* (genus *Umbravirus*) (RNA-2). The name of Holly fern mottle virus (HFMV) is proposed for this new virus species. Primers for RT-PCR detection of the virus have been developed and successfully used to test infected ferns. An increase in the number of HFMV-infected plants in various locations has been observed, suggesting that there is a natural vector for the virus. Therefore, research is being conducted to determine the means of natural spread of this virus.

Relationship between potato zebra chips symptom incidence and detections of Potato Virus Y (PVY), Potato Virus Y strain N (PVY^N) and unbalance nutritional index

L. M. VASQUEZ SILLER (2), J. Covarrubias-Ramirez (1), J. Garzon-Tiznado (1), M. Zuñiga-Estrada (1), S. Garcia-Garza (1)
(1) Inst. Nacional de Investigaciones, Forestales Agricolas y Pecuarias; (2) UAAAN
Phytopathology 98:S162

PVY and PVY^N are associated with a potato industry problem related with the process of chips frying by producing a kind of deep brown color with "zebra" pattern in tubers, and with an unbalanced nutritional index (UNI) of potato plants. This study determined the relationship between the nutritional content in potato plants during different growing stages and incidence of PVY y PVY^N, and incidence-severity of zebra chips symptom. Surveys to detect these viruses were conducted on plants at the beginning of tuberization, during the tubers growing, and on plants near maturity, in 7 potato fields in Mexico. Evaluations of incidence and severity of zebra symptoms were done at harvest by using a weighted mean obtained by comparisons with a standardized zebra symptom scale. The UNI was obtained by evaluating the difference between contents of N, P, K, Ca, Mg, Fe, Zn, Mn, Cu, Bo, and Na on leaflets and the nutrients sufficiency level registered for the potato crop. Correlations between those factors were made with Pearson method by SAS program. Incidence of PVY and PVY^N in plants was 2.4%. Correlation between such viruses and UNI was not found (0.11, Prob > [r] = 0.4). Zebra chips incidence was up to 1% in 4 out of 7 fields and in the other 3 fields ranged 8.4–20.8%. No correlation was obtained between incidence of viruses, zebra chips symptom and UNI. Results revealed that evaluated plants showed an adequate mineral nutrition, thus, it can be assumed that such nutrient balance increased the resistance of plants to viruses infections.

Relationship between the incidence of potato purple top (PPT) and Potato Leaf Roll Virus (PLRV), and the incidence of their vectors in potato fields in Mexico

L. M. VASQUEZ-SILLER (3), J. M. Covarrubias-Ramirez (1), H. Almeida-de Leon (1), A. Flores-Olivas (2), G. A. Frias-Trevino (2), A. Valdez-Oyervides (2)
(1) Inst. Nal. de Investigaciones Forestales, Agricolas y Pecuarias; (2) Univ. Autonoma Agraria Antonio Narro; (3) Universidad Autonoma Agraria Antonio Narro
Phytopathology 98:S162

PPT is a potato disease associated with the incidence of a phytoplasm, efficiently transmitted by the homopterous insect Bactericera cockerelli, causing losses about 30–95% in Mexico. PPT is a syndrome associated with several pathogens at Nuevo León, Mexico, including PLRV. Considering the high incidence of both pathogens in potato fields, this work was intended to determine these pathogens differential incidence for causing PPT on variety Escort in 2006, regarding whether conditions, the relationship with their vectors populations, and the role of potato seed-tubers in the transmission of the disease. Samples of tubers before planting, at harvest, and from plants during four potato phenological stages were analyzed with ELISA to detect PLRV, and with nested-PCR using P1/P7 and R16F2N/R16R2 primers to amplfy 16S rDNA gene and 16S-23S spatial region fragment of the phytoplasm. Populations incidence of 6 vectors genera were monitored with sticky traps in three potato fields conventionally managed; weather data were registered from stations near by the fields, and correlations between all these factors done. PLRV was no detected in tubers neither it was in leaflets. The phytoplasm was positively detected in all the fields, resulting significantly correlated with temperature, RH, precipitation and with the populations incidence of Trips spp. in one field. 2.3% phytoplasm seed-tuber infections had no significant impact in the development of the disease to cause damage in harvested tubers. This study was useful to diagnose the prevalence of the phytoplasm as a causal agent of PPT in the area of research and the role of tubers as a source of inoculum under the study conditions.

Selection and mid-scale production of a fluorescent *Pseudomonas* strain as biocontrol agent for alfalfa damping-off

P. VAZ (4), L. Quagliotto (4), M. Yanes (4), N. Bajsa (2), E. Dibar (1), H. Varela (1), N. A. Altier (3), A. Arias (4)
(1) Facultad de Ingeniería, Universidad de la República, Montevideo, Uruguay; (2) Instituto Investigaciones Biológicas Clemente Estable, Facultad de Ciencias Universidad de la República, Montevideo, Uruguay; (3) Instituto Nacional de Investigación Agropecuaria, INIA Las Brujas, Canelones, Uruguay; (4) Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
Phytopathology 98:S162

Alfalfa establishment can be improved by the suppression of seedling diseases through the application of antagonistic microorganisms. Five rhizospheric fluorescent *Pseudomonas* were selected from a collection of 701 isolates taken from three different locations in Uruguay. The strains were phenotypically characterized by *in vitro* and *in vivo* assays. The ability to produce HCN, proteases, biosurfactants and the presence of phenazines biosynthetic genes was assessed. Bioassays were performed under controlled conditions to determine the ability to promote growth and protect alfalfa seedlings from *Pythium debaryanum* damping-off. Percent emergence and total biomass of plants growing in peat substrate and in two physicochemically distinct soils were determined for the five strains. As a result of these experiments, one of the strains, C119, was selected as a potential inoculant. Two experimental designs were done to determine the concentrations of glycerol and yeast extract as carbon and nitrogen sources, respectively, to be used in the culture medium. The first approach consisted in an exploratory factorial design with a central point, which was followed by a central composite design assay. The optimal medium composition, used in further steps, contained 15 g/l glycerol and 11 g/l yeast extract. Laboratory mid-scale fermentations were carried out in order to establish the parameters to be used in greater scale production. This strain will be evaluated as a biocontrol agent in field conditions.

Occurrence of American Soybean Rust *Phakopsora meibomiae* in legumes in Puerto Rico

B. VEGA (1), C. Estevez de Jensen (1)
(1) University of Puerto Rico, Crop Protection Department, Mayagüez, Puerto Rico
Phytopathology 98:S162

In Puerto Rico, natural infection of *Phakopsora meibomiae* (Arth.) Arth. is frequently observed in different legumes growing on the island. Knowledge of the potential risk of infection of cultivated and non cultivated legumes by *P. meibomiae*, is relevant for the soybean *Glycine max* (L.) Merr. seed industry in Puerto Rico. A survey was conducted in 16 municipalities in 2006 and 2007 to study the interaction between *P. meibomiae* and different legumes. The pathogen identification was carried out by conventional Polymerase Chain Reaction (PCR), using *P. meibomiae* specific primers of the internal transcribed spacer region, and an ELISA test. Leaf tissue was observed by Scanning Electron Microscopy. *Lablab purpureus* (L.) Sweet was the legume most frequently found to be infected with *P meibomiae* (63 percent). Symptoms consisted of regular lesions around the vein of the leaf (4.1–9.2 mm in diameter), with 0.17 to 56.17 uredinia per square centimeter. Two types of lesions were observed: a reddish-brown lesion (RB), associated with low severity and less sporulation and tan reaction (TAN). In *L. purpureus*, RB lesions were observed in 12 percent of the samples while 88 percent were TAN. The average number of urediospores per uredinia ranged from 35 to 211, and their dimensions were 20.36-24.42 by 15.82-19.46 microns. DNA

extracted from leaf tissue showed amplification of a 338 bp band for *P. meibomiae*. In *Pueraria phaseoloides* (Roxb.) Benth., *Cajanus cajan* (L.) Millsp., *Canavalia gladiata* (Jack.) DC., *Mucuna pruriens* (L.) DC., *Vigna vexillata* (L.) A. Rich., an immune reaction was observed. In *Crotalaria pallida* Aiton, *Pachyrhizus erosus* (L.) Urb., *Vigna luteola* (Jack.) Benth., *Phaseolus lunatus* L. and *Macroptilium lathyroides* (L.) Urb. were found to have natural infections of *P. meibomiae*. *P. pachyrhizi* was not detected in the survey.

Description of the infection of *Phakopsora meibomiae* in legume hosts

B. VEGA (1), C. Estevez de Jensen (1)
(1) University of Puerto Rico, Crop Protection Department, Mayagüez, Puerto Rico
Phytopathology 98:S163

The american soybean rust caused by *Phakopsora meibomiae* (Arth.) Arth. was detected in Puerto Rico in 1913 on Hyacinth bean *Lablab purpureus* (L.) Sweet. There are approximately 60 legumes growing in the island that can be infected by *P. meibomiae*, which may increase the risk of spread into the soybean *Glycine max* (L.) Merr. production area. Artificial inoculation of *P. meibomiae* was carried out using 15 legumes. The inoculation was conducted in a screen house where sprinkler irrigation was provided for 48 hours after inoculation. *P. meibomiae* was confirmed using conventional polymerase chain reaction. The formation of uredinia and urediospores was observed using Light and Scanning Electron Microscopy. During the experiment average maximum and minimum temperatures in the screen house were 32.8 and 18.0°C, respectively. An immune reaction was observed on *Canavalia gladiata* (Jack.) DC., *Mucuna pruriens* (L.) DC., *Pueraria phaseoloides* (Roxb.) Benth. and *Pueraria sp*. The highest disease severity was recorded in *Phaseolus vulgaris* (L.) and *P. lunatus* (L.) when compared to the other legumes. *P. vulgaris* had 5.79 uredinia per lesion (UL) and the shortest latent period (3.23 days) whereas *P. lunatus* had 4.74 UL and a latent period of 3.29 days. The incubation period was 6.27 to 6.40 days in *P. vulgaris* and *P. lunatus* respectively. *Pachyrhizus erosus* (L.) Urb. had the lowest average number of UL with 0.64, and the smallest diameter of single uredinia (100.48 µm). *Glycine max* cv. Williams inoculated with *P meibomiae* developed symptoms 4.52 days after inoculation (latent period), infection period was 10.39 days, and the diameter of single uredinia was 108.75 µm. Infection of *P. meibomiae* in the different hosts was achieved with difficulty. Symptoms developed only when detached infected leaves of Hyacinth bean were stapled on healthy tissue. This suggests that the risk of spread the disease to soybean is minimal.

***Phakopsora pachyrhizi* (Florida isolate) urediniospore adhesion to soybean leaves**

M. VELEZ-CLIMENT (1), S. Isard (1), D. Luster (2)
(1) Pennsylvania State University, Plant Pathology Department, University Park, PA, USA; (2) USDA, ARS, FDWSRU, Ft. Detrick, MD, USA
Phytopathology 98:S163

Phakopsora pachyrhizi, the causal agent of soybean rust, has the ability of producing great quantities of urediniospores which are easily disseminated through wind and rain. When these spores land on soybean leaves and encounter ideal conditions, they produce an extracellular matrix which serves to adhere to the leaves. In order to study the adhesion process, soybean leaves (cv. Williams) were inoculated with a suspension of freshly harvested urediniospores from the Florida isolate. The samples were incubated at optimal conditions of 100% RH and 20°C. A timeline of spore adhesion was created by removing samples from the incubator at specific timepoints, ranging from 0 to 48 h after inoculation. The leaves were washed to remove unattached spores and the remaining spores were dyed with lactophenol cotton blue and counted under a light microscope. Results from 10 experimental repetitions showed that urediniospores started adhering to soybean leaves as soon as 0.5 h. Starting at 1 h, more than half of the total amount of spores counted at each timepoint had germinated. Some appressoria formation was observed at 3 h. By 6 h, 55% of the spores had large appressoria and this percentage kept increasing until 24 h; when most of the spores had germinated and penetrated the leaf. Penetration was observed in low numbers at 12 h but by 24 h, 73% of the spores counted had penetrated the leaf; which tells us that penetration occurs between 12 to 24 h. As the timepoints became longer, the amount of germinated spores increased and the amount of non-germinated spores remained fairly constant; which suggests that spore adhesion is correlated to germination.

The putative ion channel DMI1 localizes to the nuclear envelope and regulates nuclear calcium spiking during early symbiotic signaling

M. VENKATESHWARAN (3), B. K. Riely (5), E. Peiter (1), M. Otegui (4), J. Sun (6), A. B. Heckmann (6), G. Lougnon (3), A. Edwards (6), G. Freshour (2), M. G. Hahn (2), D. Sanders (1), G. D. Oldroyd (6), A. J. Downie (6), D. R. Cook (5), J. Ane (3)
(1) Biology Department, University of York, York YO10 5YW, United Kingdom; (2) Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA; (3) Department of Agronomy, University of Wisconsin, Madison, WI, USA; (4) Department of Botany, University of Wisconsin, Madison, WI, USA; (5) Department of Plant Pathology, University of California, Davis, CA, USA; (6) John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom
Phytopathology 98:S163

Two major plant-microbes symbiotic interactions are legume nodulation and arbuscular mycorrhization. Several genes required for the development of these symbioses have been cloned in model legumes. Among them, the *Medicago truncatula* DMI1 protein shares strong homologies with prokaryotic ion channels such as MthK. Two DMI1 homologs in *Lotus japonicus*, named CASTOR and POLLUX, have been localized in plastids. However, our confocal and electron microscopy by high pressure freezing and cryo-substitution method places DMI1 on both inner as well as outer nuclear membranes. The N-terminal soluble domain of DMI1 is sufficient but not necessary to target the protein to nuclear envelope. This domain is also required for the functionality of DMI1. The soluble C-terminus of DMI1 contains an RCK (regulator of the conductance of K^+) domain which in MthK acts as a calcium-regulated gating ring controlling the activity of the channel. A *dmi1* mutant lacking the entire C-terminus acts as a dominant-negative allele interfering with the formation of nitrogen-fixing nodules and abolishing the induction of calcium spikes by the G-protein agonist Mastoparan. Using both the full length DMI1 and this dominant-negative mutant protein we show that DMI1 increases the sensitivity of a sodium- and lithium-hypersensitive yeast mutant towards those ions and that the C-terminal domain plays a central role in regulating this response. We also show that DMI1 greatly reduces the release of calcium from internal stores in yeast, while the dominant negative allele appears to have the opposite effect. This work suggests that DMI1 is not directly responsible for Nod factor-induced calcium changes, but does have the capacity to regulate calcium channels in both yeast and plants.

QoI sensitivity and the prevalence of DMI resistance in NY populations of the brown rot pathogen *Monilinia fructicola*

S. M. VILLANI (1), K. D. Cox (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY 14456
Phytopathology 98:S163

Brown rot caused by *Monilinia fructicola* is an important disease concern on diversified farms and in the warmer stone fruit production regions of NY. Presently, application of sterol demethylation inhibitor (DMI) and Quinone outside inhibitor (QoI) fungicides is the only reliable means of managing fruit rot. Previously, M. fructicola DMI resistance was reported in a Western NY peach orchard. However, the statewide prevalence of DMI resistance and the *M. fructicola* genetic resistant determinant "Mona" is unknown. The sensitivity of NY *M. fructicola* populations to Pristine fungicide (QoI component), widely used for fruit rot control, is also unknown. We examined 236 isolates (baseline population excluded n = 35) from 6 NY counties from 2005 to 2007. Isolates were tested for DMI sensitivity (propiconazole and fenbuconazole) using poison plate assays at discriminatory doses 10 and 100 times the previously established baseline EC_{50} concentrations. PCR-RFLP analysis for the presence of Mona was also performed. For each isolate, a dose response curve was generated for the components of Pristine fungicide alone and in combination. All isolates appeared to be shifted toward DMI resistance with stronger shifts in 2007 isolates compared to 2005 isolates. Mona was detected in two populations and represented half of the population sampled. Most populations seem to be shifted in sensitivity to the components of Pristine fungicide, but no resistant phenotypes were detected.

Screening for disease resistance to *Verticillium dahliae* in spinach

M. I. VILLARROEL-ZEBALLOS (1), L. J. du Toit (2), J. C. Correll (1)
(1) University of Arkansas; (2) Washington State University
Phytopathology 98:S163

Verticillium wilt, caused by *Verticillium dahliae*, has recently been shown to be a disease of spinach. However, infected plants rarely show symptoms until after flowering (bolting) has been initiated. As a result, Verticillium wilt is more of a concern during spinach seed production. The objectives of this research were to evaluate the reaction of spinach germplasm accessions to *V. dahliae*, and to evaluate the seed infestation/infection levels of the USDA spinach seed germplasm collection. A total of 120 spinach accessions from the USDA spinach germplasm collection and 10 commercial spinach cultivars were evaluated for resistance in greenhouse pathogenicity tests 2006, 2007, and 2008. Each spinach genotype was grown in potting mix either infested or non-infested with *V. dahliae*. Disease severity ratings were conducted weekly starting 1-week prior to bolting to 4-weeks after bolting. Results showed a

wide range in disease severity ratings among the genotypes evaluated. However, most spinach lines tested were highly susceptible to Verticillium wilt. No qualitative resistance (or immunity to infection) was identified among the germplasm tested, but five genotypes had high levels of quantitative resistance. *V. dahliae* caused a general reduction in biomass compared to plants grown in uninfested soil. Additionally, preliminary results indicated that approximately 34% (20/59) of the USDA germplasm seed lots tested were infected/infested with *V. dahliae*.

Physic nut diseases

N. VISARATHANONTH (1), L. Manoch (1)
(1) Dept. of Plant Pathology, Kasetsart University, Bangkok 10900, Thailand
Phytopathology 98:S164

Physic nut (Jatropha curcas) is a field crop which extracted oil from seeds are directly used as biodiesel for agricultural farm engines. Area of planting is increasing in Southeast Asian countries. Disease surveys of Physic nut or Jatropha were conducted from different locations of Provinces in Thailand and were mostly due to many fungal pathogens. Leaf spots are mainly caused by Cercospora sp., while, the other minor observed are Pestalotiopsis sp. and Macrophoma sp. Anthracnose disease is observed on both Jatropha fruits and leaves is due to Colletotrichum gloeosporioides. Powdery mildew disease caused by Oidium sp. is newly observed on leaves, petioles, shoots and different stages of fruit growth, resulting russetting fruit peels. It might reduce both quantity and quality of oil extracted from thus infected Jatropha seeds. Die-back disease caused by Schizophyllum commune is another disease entering through wounds of cutting practices. Many suspected symptoms and unidentified specimens are being more observed. Jatropha diseases are needed to report for further control measures for oil yields increasing that may concern on diesel oil substitute in the near future.

A mathematical model for Carlavirus disease incidence on experimental soybean plots in Puerto Rico

D. M. VITERI (1), L. Gordillo (2), C. Estevez de Jensen (1)
(1) University of Puerto Rico, Crop Protection Department, Mayagüez, Puerto Rico; (2) University of Puerto Rico, Department of Mathematics, Mayagüez, Puerto Rico
Phytopathology 98:S164

Stem necrosis is a recently observed disease in soybeans [(*Glycine max*) (L.) Merr.] in Puerto Rico. The disease is caused by a whitefly-transmitted Carlavirus and in susceptible cultivars the disease can be severe. Our aim was to analyze both vector and the disease dynamics. A mathematical model was developed to understand the Carlavirus incidence progression. Experimental plots were planted with soybean line 2053A in two locations. Vector population and disease incidence evaluations were conducted in 2006 and 2007. Carlavirus disease incidence was modeled as a compartmental epidemic, which takes into account the non-persistent nature of the virus. Several assumptions were made, including ignoring latency periods in the host, effects of cyclic planting and harvest and hosts acquiring the virus only through the feeding of infected vectors which turn susceptible. The base-line data of the whitefly (*Bemisia tabaci*) population in the second week was 25,000 and 40,000 adults/acre in Juana Diaz and Isabela, respectively. Disease symptoms were observed 20–30 days after planting (DAP) (R1 soybean stage), as leaf vein and stem necrosis. Incidence increased to 5 percent (65 DAP) and from the onset of the disease plant stunting and death occurred. In a second experiment, disease incidence increased from 1 (30 DAP) to 10 percent (70 DAP). Analysis of the model revealed that soybean monoculture and high withefly population at the beginning of the crop cycle cause the increase of disease incidence. The proposed model can be valuable for evaluating the need for vector control measures, in order to obtain consecutively decreasing Carlavirus incidences in the host plant population.

Improving survival of beneficial bacteria on anthurium leaves to control bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae*

T. S. Vowell (1), P. J. TOVES (1), A. M. Alvarez (1)
(1) University of Hawaii, Honolulu, HI 96822
Phytopathology 98:S164

A consortium of beneficial bacterial species (*Sphingomonas chlorophenolica*, *Microbacterium testaceum*, *Brevundimonas vesicularis* and *Herbaspirillum rubrisubalbicans*) are used as biological control agents (BCAs) of anthurium blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Xad). Maintaining high populations of the BCAs on anthurium leaves is essential to effective biological control. BCA survival on *Anthurium antioquiense* and *A. andraeanum* microplants was assessed up to four weeks after spraying leaves with BCAs at 10^9 colony forming units/ml. Populations of *S. chlorophenolica* and *M. testaceum* did not survive as well on *A. andraeanum* as on *A. antioquiense* decreasing by 2-3 and 5-6 log units, respectively, on *A. andraeanum* compared to the initial inoculum. In contrast, populations remained fairly constant on *A. antioquiense* for 3 to 4 weeks for all BCA strains except for *H. rubrisubalbicans*, which dropped by 6-7 log units compared to the initial inoculum. Similar survival trends were observed when BCAs were collected from the guttation fluid of large *A. antioquiense* plants. Addition of surfactants (Silwet L-77 and Tween-20) neither improved survival of BCAs nor reduced the amount of foliar disease. On the other hand, foliar applications of selected amino acids in combination with BCAs, while not affecting BCA populations in guttation fluids, significantly decreased disease severity.

What are we waiting for? Lunar crop science: The final frontier

C. H. WALKINSHAW (1)
(1) USDA Forest Service, Pineville, LA, USA
Phytopathology 98:S164

In highly controlled tests, botanical tissues displayed significant variability in their response to lunar soils collected on several consecutive Apollo missions. Germfree environments permitted measurements without microbial contamination. Physiological and chemical measurements created a definition of the botanical suitability of lunar soils. In this presentation, the results of numerous replicated tests are summarized. These unique data strongly indicate lunar crop science as a novel, and fruitful area of plant pathology.

Symptoms and signs of *Stigmina lautii* on spruce needles in North Dakota

J. A. WALLA (1), K. M. Kinzer (1)
(1) North Dakota State University, Fargo, ND, USA
Phytopathology 98:S164

Stigmina lautii Sutton has recently been associated with needlecast of spruce in ND, but has not been proven a pathogen. The objective of this research was to characterize symptoms and signs associated with *S. lautii* and to compare them with those of *Rhizosphaera kalkhoffii* Bubak. Spruce with needlecast were observed and fungal fruiting bodies on needles were examined microscopically to determine identity. Both fungi appear to cause greatest needlecast in the lower canopy and on the north side of a tree. Discoloration associated with both fungi may develop on second-year or older needles and may be grayish-green, yellow, tan, brown, reddish-brown or purple. Both Colorado and white spruce appear to be severely affected by *S. lautii*, while only Colorado spruce is commonly damaged by *R. kalkhoffii* in ND. *S. lautii* was generally present on second-year needles of all affected trees, while *R. kalkhoffii* varies from tree to tree in age of youngest needles affected. Fruiting bodies of both fungi can occur on green, discolored, and dead second-year and older needles, are similar in size and color, and cannot be distinguished without magnification of 20X or more. Microscopically, side-protruding conidia of *S. lautii* sporodochia give a feathery appearance to the fruiting bodies compared to the smooth *R. kalkhoffii* pycnidia. In ND samples, the brown *S. lautii* spores averaged 41.7 µm long, 3.2 µm wide at the narrow end, and 5.2 µm wide at the wide end. Most conidia (65%) had 3 to 5 septa. *R. kalkhoffii* spores are hyaline, smaller (4.5-8.6 × 2.5-4.6 µm), and aseptate. Because symptoms and signs associated with *S. lautii* and *R. kalkhoffii* on spruce appear superficially similar, microscopic confirmation is required to identify which fungus is present.

The root endophytic fungus *Piriformospora indica* accelerates host plant development and primes plants for disease resistance

F. WALLER (1), A. Molitor (1), S. Pfiffi (1), B. Achatz (1), K. Kogel (1)
(1) Institute of Phytopathology, Justus-Liebig-University Giessen, Heinrich-Buff-Ring 26-35, D-35392 Giessen, Germany
Phytopathology 98:S164

The beneficial basidiomycete *Piriformospora indica* colonises the roots of plants of a wide range of phylogenetic groups, e.g. barley (*Hordeum vulgare* L.) and *Arabidopsis thaliana*. Endophytic growth is restricted to the roots of the host plant and fungal development positively correlates with cell death, while inducing higher growth rates in the host tissue. *P. indica* induces (1) enhanced resistance against the biotrophic powdery mildew fungus *Blumeria graminis* f. sp. *Hordei* and (2) increases grain yield. Detailed analysis of yield parameters revealed that the grain yield increase is independent of low or high soil P and N supply, which is in contrast to arbuscular mycorrhizal fungi. *P. indica* colonised barley developed roots and shoots faster early in development, resulting in the formation of more tillers and ears per plant leading to higher grain yield. To evaluate the mechanism of increased systemic resistance, we analysed the barley root and leaf transcriptome using the Affymetrix Barley-1 GeneChip. Gene expression in the leaves of *P. indica*-colonised and non-colonised barley plants was compared 12, 24 and 96 h after *Blumeria graminis* infection. Specific expression of a set of genes 12 h after challenge with *B. graminis* in *P. indica*-colonised plants was detected. We will present detailed expression data and functional annotation of gene candidates supporting a role of *P. indica* in priming plants for enhanced resistance.

Evaluation of a soil baiting technique to test the efficacy of fungicidal seed treatments against soybean seedling pathogens

M. W. WALLHEAD (1), K. D. Broders (1), M. L. Ellis (1), A. E. Dorrance (1)
(1) The Ohio State University, OARDC, Wooster, OH
Phytopathology 98:S165

Soybean seedling disease is caused by a number of different pathogens representing several taxonomic groups. The development of soybean seedling disease is very dependent on the presence of these plant pathogens and the environment. Soybean fungicide seed treatments can be very effective in managing many soil borne pathogens. The objective of this study was to evaluate the efficacy of fungicide seed treatments on soybean using a greenhouse assay with field soil. Treatments include; a non-treated control, Apron Maxx (mefenoxam 1.1% + fludioxonil 0.73%), Apron XL (mefenoxam 32.3%), Apron Maxx+Aprox XL (mefenoxam 1.1% + fludioxonil 0.73%, mefenoxam 32.3%), Captan 400 (Captan 37.4%), and Maxim 4S (fludioxonil 40.3%). Stands for the non-treated control were significantly lower than all of the fungicide treatments from soil collected from one problem field. This indicates that this greenhouse assay may be useful to assess efficacy of seed treatment fungicides on natural pathogen populations.

Are terpenes involved in Austrian pine (*Pinus nigra*) resistance to the fungal pathogen *Diplodia pinea*?

C. M. WALLIS (2), P. Bonello (1)
(1) Ohio State University, Columbus, OH; (2) University of Northern British Columbua, Prince George, BC, Canada
Phytopathology 98:S165

Diplodia pinea is a serious tip blight pathogen of Austrian pine (*Pinus nigra*), a commonly planted landscape tree. The aim of this work was to relate changes in terpenoid levels with the expression of local and systemic induced resistance to *D. pinea*. The stem base of potted, 4-yr-old trees was inoculated with either *D. pinea* or *D. scrobiculata* (a less aggressive sister species). After incubating the trees for 21 days in a greenhouse, phloem tissues and resin samples, which were exuded from stem wounds, were collected 5 or 30 cm from the inoculation site. Controls consisted of mock-inoculated and uninoculated trees. All pathogen induced and non-induced trees were challenged with *D. pinea* at this point in time 30 cm above induction site, and resulting challenge lesion lengths were measured after 14 more days to assess systemic induced resistance to the pathogen. Terpenoids were extracted in dichloromethane and analyzed by gas chromatography. Both oleoresin and phloem terpenoid levels responded similarly to the induction treatments. Levels of alpha-pinene, beta-pinene, camphene, limonene, and myrcene increased around a *D. pinea* or *D. scrobiculata* infection. Levels of beta-pinene, myrcene, limonene, and bornyl acetate decreased systemically in trees infected with *D. pinea*, but appeared to increase systemically in trees infected by *D. scrobiculata*. Pre-challenge systemic terpenoid levels were not positively correlated with the resulting expression of resistance, but levels of terpenes post-challenge, around the challenge infection, indicated that higher terpene levels were associated with reduced lesion lengths. These results suggest that terpenes act to limit infection progress in this pathosystem.

Allele mining for genes associated with partial resistance to *Phytophthora sojae* in soybean

H. WANG (3), S. Berry (3), S. K. St. Martin (2), L. Zhou (4), K. Krampis (4), D. Tucker (1), Y. Mao (4), I. Hoeschele (4), M. Maroof (1), B. Tyler (4), A. E. Dorrance (3)
(1) Crop and Soil Environmental Science, Virginia Tech, Blacksburg, VA; (2) The Ohio State University, Columbus, OH; (3) The Ohio State University, OARDC, Wooster, OH; (4) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA
Phytopathology 98:S165

Phytophthora sojae causes soybean root and stem rot, resulting in an annual loss of 1–2 billion dollar in soybean production worldwide. Partial resistance confers a broad-spectrum durable resistance to *P. sojae* and serves as a more stable alternative than single gene mediated resistance. However, little is known about the molecular mechanisms behind it. In this study, microarray analysis was applied for global expression profiling of over 37500 transcripts in soybean during *P. sojae* infection, on a partial resistant soybean cultivar 'Conrad' and a susceptible cultivar 'Sloan'. The contrast analysis revealed ~500 genes having significantly different expression pattern in 'Conrad' at 3 dai (day after inoculation) and ~1000 at 5 dai ($P < 0.05$), which implicates their potential roles in partial resistance. Four potential QTLs in MLGs F, H, J and L were identified in 'Conrad' from the 186 RILs of $F_{4:6}$ 'Conrad' × 'Sloan' population by single marker association ($P < 0.01$). These results infer that partial resistance in 'Conrad' is a complex trait that involves numerous genes whose expression is influenced by at least four loci.

The Rcs phosphorelay system is essential for pathogenicity in *Erwinia amylovora*

D. WANG (1), Y. Zhao (1)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL 61801
Phytopathology 98:S165

The Rcs phosphorelay system (RcsCDB) is a modified two-component signal transduction system (TCST) found exclusively in Enterobacteriaceae. The Rcs system, first identified by its role in regulating genes for capsular polysaccharide in *E. coli*, turns out to be a very complicated TCST and is involved in many cellular responses such as cell motility and attachment. *Erwinia amylovora* is a highly virulent and necrogenic enterobacterium causing fire blight disease on rosaceous plants. Amylovoran, an exopolysaccharide, is one of the two major pathogenicity factors in *E. amylovora*. In this study, we characterized the roles of the Rcs system in *E. amylovora*. Our results showed that rcsB, rcsC, rcsD and rcsBD mutants were non-pathogenic on immature pear fruit. Bacterial growth of these mutants was also greatly reduced compared to that of the wild type (WT) strain in immature pear fruit. In an *in vitro* amylovoran assay, rcsB and rcsD mutants were deficient in amylovoran production, whereas rcsC mutant showed an increased amylovoran production than that of the WT. In consistency with amylovoran production, gene expression of the amylovoran biosynthetic gene amsG, using GFP as a reporter, was not detectable in rcsB, rcsD and rcsBD mutants *in vitro*. However, amsG was expressed much higher in rcsC mutant than that of the WT. We also found that the rcs mutants were more susceptible to polymyxin B treatment than that of WT, suggesting that the Rcs system confers the bacterium resistance to polymyxin B. Furthermore, the rcs mutants showed irregular and reduced swarming motility on swarming plates containing 0.3% agar. These results indicate that the Rcs system is a pathogenicity factor and also plays a major role in the survival of *E. amylovora*.

Membrane associated stigmasterol plays an important role in plant innate immunity

K. WANG (2), C. Ryu (1), L. Kang (2), S. Muthappa (2), K. S. Mysore (2)
(1) Korea Research Institute of Bioscience and Biotechnology, Yuseong, Daejeon, South Korea; (2) Samuel Roberts Noble Foundation, Ardmore, OK, USA
Phytopathology 98:S165

Plant nonhost resistance, a form of innate immunity, is the most common form of disease resistance exhibited by plants against the majority of potential pathogens in nature. We used virus-induced gene silencing in *Nicotiana benthamiana* to identify genes involved in nonhost resistance. We individually silenced ~4,000 genes by using cDNA clones from a normalized NbcDNA library. Eleven genes were identified to be involved in type I and/or type II nonhost resistances. One of them encodes squalene synthase (SQS), a key enzyme catalyzing the first enzymatic step in sterol biosynthesis. The *Arabidopsis SQS1* RNAi lines were not only susceptible to nonhost pathogens, *Pseudomonas syringae* pv. *tabaci* and *P. syringae* pv. *syringae*, but also more susceptible to pathogens, *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *maculicola*, when compared to wild-type *Arabidopsis*. We also discovered that a mutation in *Arabidopsis SMT2*, a gene encoding sterol methyltransferase (downstream enzyme in phytosterol biosynthesis), also compromised nonhost resistance. Silencing *SQS* gene resulted in plant cell membrane leakage in both *Arabidopsis* and *N. benthamiana*. Metabolite analysis indicated that, compared to the wild-type *Arabidopsis*, *SQS1* RNAi lines and a *smt2* mutant produced less stigmasterol. Strikingly, we found more stigmasterol accumulation in the wild-type plants upon inoculation with a nonhost pathogen. Our data suggest that the membrane associated stigmasterol plays an important role in plant immunity against bacterial infections.

Alfalfa common leaf spot pathogen and its effects on related enzymatic activity of the host plant

J. Wang (1), L. ZHOU (1)
(1) China Agricultural University
Phytopathology 98:S165

The diseases of alfalfa (*Medicago sativa* L.) have become more and more serious recently which lead to decline of grass output and quality. The alfalfa common leaf spot is one of the most popular and serious diseases in China. The diseased leaves were collected in early September 2006 at Guyuan in Ningxia of China. The pathogen was obtained successfully by using different separating methods, and was identified as Pseudopeziza medicaginis by both morphological and molecular characteristics. It was found that there were two types of colonies which color was either black or pink. Otherwise, there were a lot of lipid droplets exuded from the mycelia. Single spores were obtained by the ascospore ejecting on medium surface from the matured asci. Alfalfa juice, tomato juice, celery juice, carrot juice media were screened to be appropriate media for both mycelia growth and sporulation. After the pathogen was inoculated to either compatible cultivar race (i.e. Rambler) or

incompatible one (i.e. Polato), disease-resistance related enzymatic activities of phenylalanine ammonia lyase (PAL), peroxidase (POD), superoxide dismutase (SOD), beta-1,3-glucanase were detected to have correlation with cultivar disease resistance.

Postharvest *Aspergillus flavus* colonization in responding to preharvest field condition of drought stress and oligo-macroarray profiling of developing corn kernel gene expression under drought stress

Z. WANG (4), J. Liu (1), D. Lee (3), B. Scully (2), B. Guo (2)
(1) J. Craig Venter Institute, Rockville, MD; (2) USDA-ARS, Crop Protection and Management Unit, Tifton, GA; (3) University of Georgia, Department of Crop and Soil Sciences, Tifton, GA; (4) University of Georgia, NESPAL, Tifton, GA
Phytopathology 98:S166

Drought stress is a major factor to enhance preharvest aflatoxin contamination of maize kernels. *Aspergillus flavus* infection of maize kernels occurs earlier than aflatoxin accumulation in developing kernels. Recent studies have demonstrated higher concentration of defense or stress-related proteins in maize kernels of resistant genotypes than susceptible genotypes, suggesting that preharvest field condition influences gene expression differently in different genotypes resulting in different levels of "end product"-PR-proteins. Our goal is to study preharvest gene activities and effects on postharvest fungal colonization and aflatoxin production. The experiment was conducted in the field cages with irrigation system. Total 48 diverse inbreds were planted. Controlled pollination was done manually. Drought stress was imposed at 18 days after pollination (DAP) for stressed treatment. Immature ears were collected at 35 DAP for gene expression analysis. Mature ears were hand harvested and tested for postharvest A. flavus colonization and aflatoxin production in the laboratory. The fungal colonization of kernels and aflatoxin concentration suggest that preharvest drought stress could affect on postharvest host-A. flavus interactions differently in different genotypes, suggesting that different inbred lines with different levels of drought tolerance respond differently to the field condition. Preharvest drought stress increased or decreased postharvest fungal colonization and aflatoxin production. Gene expression analyses of preharvest developing kernels by using oligounclrodide macroarray of 211 selected genes identified 72 genes significantly up- or down-regulated by drought stress in comparison with irrigation. Sequences homology analysis showed 92% have known functions in constitutive or inducible defense mechanism, and only 8% with functions unrelated to defense or unknown. These results indicate that the genetics of individual line is different in response to drought stress, and maize plants not only regulate general stress-related genes, but also induce specific antifungal genes in reaction to the stress and result in the observed increased or decreased resistance response.

High-resolution genetic and physical mapping of the *Yr5* gene for resistance to stripe rust of wheat

M. WANG (1), T. Coram (3), P. Ling (3), L. Boyd (2), X. Chen (3)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, USA; (2) John Innes Centre, Norwich, England, UK; (3) USDA-ARS and Department of Plant Pathology, Washington State University, Pullman, WA, USA
Phytopathology 98:S166

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a destructive disease of wheat worldwide. The *Yr5* gene confers resistance to all races of the pathogen identified so far in the U.S. To clone *Yr5*, an F_2 segregating population consisting of 1400 plants from a cross between the susceptible cultivar 'Avocet S' and a *Yr5* resistant near-isogenic line (NIL) in the 'Avocet S' background was phenotyped for stripe rust reactions using race PST-43. A fine genetic map of the *Yr5* locus was constructed using resistance gene analog polymorphism (RGAP), sequence tagged site (STS), and bacterial artificial chromosome (BAC) end markers. These markers spanned the *Yr5* locus within a 0.7cM genetic interval. The most closely linked marker was 0.14cM from the *Yr5* locus. The genomic BAC library of the *Yr5* NIL was screened using the flanking markers STS7/8, STSuk1, and STSuk2. Six BAC clones were identified by the two closest markers and three more BAC clones were identified by one step of chromosome walking towards the *Yr5* locus. The nine BAC clones consist of a physical contig of about 400 kb spanning the *Yr5* locus corresponding to a 0.19 cM genetic interval. Three RGAs were identified in this region, which were highly homologous with resistance proteins in rice chromosome 4 belonging to the nucleotide binding site-leucine rich repeat (NBS-LRR) class. The results indicate that *Yr5* is located in a resistance gene cluster on chromosome 2BL.

Phytophthora species associated with silver maple bleeding canker in northern Nevada

S. WANG (2), L. Lyles (1), S. Garneni (2), W. J. Carlos (3), P. McKie (2)
(1) Certified Aroborist, Reno, NV, USA; (2) Nevada Department of Agriculture, Reno, NV, USA; (3) University of Nevada, Reno, NV, USA
Phytopathology 98:S166

A bleeding canker disease, symptomatically similar to sudden oak death, was first noticed in 1999 on silver maple trees (*Acer saccharinum*) in northern Nevada. The disease has caused decline or loss of both young and mature trees. In an effort to identify the cause, pieces of fresh phloem and xylem tissue were collected from the margin of lesions underneath the canker area, and then placed on pimaricin-ampicillin-rifampicin-PCNB agar to isolate *Phytophthora* species. Six isolates (SM1-SM6) obtained from different locations represent two groups morphologically. Five of them (SM2-SM6) have identical morphology similar to *P. cactorum*, and one (SM-1) differs from other isolates. To confirm their identities, regions of rDNA including partial 18S ribosomal RNA gene, ITS1, 5.8S ribosomal RNA gene, ITS2 and 28S ribosomal RNA gene were amplified from selected isolates, subcloned into pGEM®-T vector, and then sequenced using T7 promoter and SP6 upstream primers. DNA sequences of *P. cactorum*-like isolates generated a minimum of first 97 hits of deposited *P. cactorum* sequences and had 99% nucleotide identities by a BLAST search of the GenBank database. DNA sequence of SM-1 matches mostly the sequences of 7 isolates of an undescribed *Phytophthora* species followed by 74 hits of deposited *P. citricola* sequences. Thus, the identities of these isolates were confirmed as *P. cactorum* and an undescribed *P. citricola*-related species. Both species are believed to be the primary cause of silver maple bleeding canker in northern Nevada.

Effects of two cover crops on nematode communities in Helicotylenchus multicinctus infested banana fields

K. WANG (1), C. R. Hooks (1)
(1) University of Hawaii
Phytopathology 98:S166

Recently the spiral nematode, Helicotylenchus multicinctus, was found in great abundance and frequency on banana (Musa spp.) plantings throughout Hawaii. Objectives of this study were to determine if planting French marigold, Tagetes patula cv. Ground Control or sunn hemp, Crotalaria juncea cv. Tropic Sun could suppress spiral nematodes, while enhancing beneficial nematodes in the soil. Soil collected from two H. multicinctus infested banana fields from Lanai City and Waianae, HI were potted in 8-cm diameter clay pots in a greenhouse and planted with 'Santa Caterina' banana, sunn hemp or marigold. Initial and final (5 months after planting) population densities of H. multicinctus and omnivorous and predatory nematodes were determined. Both sunn hemp and marigold supported lower ($P < 0.05$) numbers of H. multicinctus than banana in both soils. In Waianae soil, population densities of omnivorous and predatory nematodes were found in greater ($P < 0.05$) abundance in soil planted with marigold than those planted with banana and sunn hemp at the end of the experiment. No treatment was found different in the number of predatory and omnivorous nematodes in Lanai soil. We conclude that marigold 'Ground Control' is an ideal cover crop for managing H. milticinctus and potentially a good cover crop to enhancing beneficial nematodes in banana fields.

First report of maize bacterial stalk dry rot in China

X. WANG (1)
(1) Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China
Phytopathology 98:S166

Since 2005, a serious stalk disease has occurred on the paternal parent (PS056, male plant) up to 80% to 100% plants in maize (Zea mays L.) hybrid seed production fields in Xingjiang and Gansu Provinces, China. Because of slow growth and shortening in plant height, the pollens of the male plants were not able to spread onto the silks of the female plants, resulting in severe reduction of seed yields. The typical symptoms on the infected plants are shortened nodes with irregular and brown spots on the leaf sheaths. At the lower parts of the stalks, the pith and vascular tissues were brown and dry rot. At later stage, an irregular breach was formed with dissolving of certain diseased hard bark and pith tissues. The spots were developed rapidly in one side of the infected stalks. The diseased tissues became brittle and the plants were easily broken from the infected parts of the stalks. These symptoms of the disease were different from bacterial stalk rot, which is caused by Erwinia chrysanthemi pv. zeae. The plants that suffer from bacterial stalk rot show brown, soft, and water soaking tissue with unpleasant odor on stalks. Pathogenicity test and inoculation, identification of isolates on morphology, cultural and biochemical characteristics, analysis of fatty acid by Sherlock® microbial identification system, and sequencing of 16S rRNA gene were carried out to characterize the pathogen isolations. The causal agent of this bacterial stalk dry rot disease was determined to be the bacterium Pantoea agglomerans (syn. Erwinia herbicola). This is the first report of a new disease, bacterial stalk dry rot, in maize.

Streptomyces scabies populations in a single field are not clonal and shift from year to year

L. A. WANNER (1)
(1) USDA-ARS, Beltsville, MD, USA
Phytopathology 98:S167

Molecular typing methods are easily devised that distinguish strains within individual microbial species, enabling researchers to follow the origins, evolution and spread of pathogens within and between hosts and environments. To study the evolution and population dynamics of plant pathogenic *Streptomyces* species in the field, PCR primer sets were devised with one primer anchored in an insertion sequence element found in *S. scabies*, *S. acidiscabies* and *S. turgidiscabies*. These primer sets and repetitive sequence element (BOX) PCR were used to fingerprint several hundred *S. scabies* isolates collected over seven years from a common scab field in northern Maine. All isolates shared markers for the *S. scabies* pathogenicity island, and belonged to species *scabies*, as determined by 16s ribosomal DNA type. While molecular forensics studies indicate that epidemics of *Salmonella* in humans and animals are associated with single clonal pathogen populations that prevail over large geographical areas, the data from the Maine scab field show as many as 10 distinct pathogenic *S. scabies* strains could be distinguished in a single field/year, and strain profiles shift dramatically from year to year. Multilocus sequence typing is being developed to independently corroborate strain designations, and strain profiles are being compared to those from nearby and distant areas. The broad aim is to determine the origin of strain variation and its ecological and epidemiological significance. This information can be applied to examine the relationships between pathogenic and non-pathogenic soil isolates, and to determine whether plant pathogenic *Streptomyces* causing potato common scab are spread via seed potatoes or are endemic to soils.

Characteristics of whitefly transmission of Squash vein yellowing virus

S. E. WEBB (3), S. Adkins (2), C. A. Baker (1)
(1) Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL; (2) USDA-ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL; (3) University of Florida, Entomology and Nematology, Gainesville, FL
Phytopathology 98:S167

Squash vein yellowing virus (SqVYV), a recently described ipomovirus, is transmitted by the whitefly, *Bemisia tabaci* (Gennadius), B strain. Understanding the characteristics of transmission is essential for developing management strategies for this virus, which is the causal agent for watermelon vine decline in Florida. Laboratory and greenhouse experiments examined transmission efficiency and the effect of inoculation and acquisition access times. Whiteflies were added to a cage containing SqVYV-infected plants for specific acquisition access periods (AAP) and then collected and placed in clip cages for inoculation access periods (IAP) on uninfected squash seedlings (25 or 30 per treatment). At least 30 whiteflies per plant were required to obtain an average of 46% infection in three experiments (24-h AAP and IAP). In a twice-repeated experiment with 30 whiteflies per plant and a 24-h AAP, no plants became infected after a 30-min IAP. IAPs of 2 and 4 h resulted in similar rates of transmission (12 and 16%), with a doubling of transmission after 8 h (32%). In contrast, in one experiment, AAPs of 1, 2, 4, 8, and 24 h resulted in 47, 33, 73, 80, and 20% transmission respectively after a 24-h IAP with 30 whiteflies per plant. The decreased rate of transmission after 24 h is being examined further. Rapid acquisition of this virus by whiteflies could lead to rapid spread in field situations if feeding adults move to new plants after disturbance by cultural practices and harvesting.

Fungal species colonizing ethanol fermentation co-products

C. WEBER (2), C. Engelbrecht (1), G. Munkvold (2)
(1) Iowa State University, Dept. of Plant Pathology, Ames, IA, USA; (2) Iowa State University, Seed Science Center, Ames, IA, USA
Phytopathology 98:S167

The recent rapid expansion in ethanol production has resulted in an increase in ethanol co-products available as livestock feed components. Consumption of ethanol co-products may have health implications for livestock, due to mycotoxin exposure. Mycotoxins in grain typically become concentrated in the co-products during the fermentation process; additionally, new fungal colonization of co-products is likely to occur during transport and storage. Our study sought to identify potential mycotoxin-producing fungi present in samples of ethanol co-products, including wet distillers' grains (WDG), dried distillers' grains & solubles (DDGS), and condensed distillers' solubles (CDS). Samples of co-products were collected from two locations from Oct 2006 through Mar 2007 and dilutions were cultured on semi-selective media for fungi. Colony counts were performed and representative colonies were isolated and identified by morphological characters, carbon-source utilization (Biolog® analysis), and ITS sequences. Fungal populations ranged from 2.00×10^4 cfu/g to 2.10×10^7 cfu/g. CDS primarily contained various yeasts, while filamentous fungi were common in the other co-products. In general, higher populations and more diverse microflora were found in WDG compared to DDGS. The most common filamentous fungi were several species of *Penicillium*, including *P. roquefortii* and *P. crustosum*. Other common fungi included *Aspergillus flavus* and other *Aspergillus* species, *Cladosporium* sp., *Mucor circinelloides*, as well as various yeast species. *Fusarium* spp. were recovered but were not predominant. The frequent occurrence of potential mycotoxin-producing *Penicillium* and *Aspergillus* species indicates the need for monitoring mycotoxin levels found in ethanol co-products, especially WDG. In addition, more detailed studies are needed on fungal growth rates and mycotoxin development over time in each type of co-product in order to better understand spoilage issues during transport and storage.

Evaluation of fungicide seed treatments for control of sudden death syndrome of soybean

J. D. WEEMS (1), G. Zhang (1), K. A. Ames (1), C. A. Bradley (1)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL, USA
Phytopathology 98:S167

Sudden death syndrome (SDS) of soybean (*Glycine max*), caused by *Fusarium virguliforme*, is present throughout the state of Illinois, and can cause yield losses when environmental conditions are favorable. Although soybean cultivars vary in their susceptibility to SDS, no cultivars have been developed that have complete resistance. Recent research has indicated that *F. virguliforme* can infect soybean plants as soon as the radicle begins to emerge from the seed, which would suggest that fungicide seed treatments may be able to provide some protection against *F. virguliforme* during these early stages. A field study was initiated in 2007 at Urbana, IL to evaluate the effect of fungicide seed treatments on SDS. Sixteen fungicide seed treatments and an untreated control were evaluated on soybean planted into a field that was artificially infested with *F. virguliforme* growing on sterilized grain sorghum seed. To track the infection of *F. virguliforme*, quantitative PCR (Q-PCR) was used with DNA extracted from roots collected at three different times during the growing season. Foliar symptoms of SDS were rated, and harvested grain yield was measured. On DNA extracted from the first root collection date, Q-PCR analysis indicated that the cycle threshold (Ct) ranged from 32 to 37 cycles (out of 40 cycles total), with the untreated control at 32 cycles; however, these did not differ significantly ($P \leq 0.05$). Overall, SDS severity as measured by foliar symptoms were low. No significant differences were observed among treatments for SDS severity or yield.

Evaluation of visual and optical sorting of Fusarium damaged kernels in winter wheat

S. N. WEGULO (2), F. E. Dowell (1)
(1) USDA-ARS, Manhattan, KS, USA; (2) University of Nebraska-Lincoln, NE, USA
Phytopathology 98:S167

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, often results in shriveled and/or discolored kernels referred to as Fusarium damaged kernels (FDK). Determination of FDK usually is done visually. Visual sorting can be laborious and is subject to inconsistencies resulting from variability in intra-rater repeatability and/or inter-rater reliability. Visual sorting of FDK in 21 subsamples of scabby wheat grain by two raters was compared to sorting by a single kernel near infrared (SKNIR) system. Visual sorting was strongly correlated with sorting by the SKNIR system ($0.82 \leq r \leq 0.91$). However, the SKNIR system was more consistent with correlation coefficients between replicate runs (4 runs) in the range $0.91 \leq r \leq 0.96$ compared to $0.49 \leq r \leq 0.80$ for visual sorting. Standard errors (SE) of the mean of FDK sorted by the SKNIR system were smaller ($0.82 \leq SE \leq 7.09$) than SE of the mean of FDK sorted visually ($2.63 \leq SE \leq 16.78$). The results from this study indicate that consistency can be improved by sorting FDK with the SKNIR system.

Relating yield loss to tan spot severity in winter wheat

S. N. WEGULO (1), J. A. Breathnach (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 98:S167

Tan spot of wheat, caused by *Pyrenophora tritici-repentis*, is an important disease of wheat worldwide. Yield losses of up to 50% from the disease have been reported. In 2007, field experiments were conducted in Nebraska, USA on winter wheat cultivar Millennium to establish relationships between tan spot severity and yield loss at four locations. Conditions were favorable for tan spot at all locations. Different levels of disease severity were established in rain-fed field plots by varying the timing (Feekes 6 or 9) and number (1 or 2) of applications of the fungicides azoxystrobin, pyraclostrobin, propiconazole, azoxystrobin + propiconazole, and trifloxystrobin + propiconazole. Regression of yield loss on disease severity at flowering resulted in the strongest relationship between the two variables ($0.69 \leq R^2 \leq 0.92$, $P < 0.0001$). Every 1% increment in disease severity at flowering resulted in 0.8,

0.4, 1.3, and 0.5% yield loss at Mead, Clay Center, North Platte, and Sidney, respectively. These results suggest that yield loss from tan spot may vary with location.

Causes and prevention of Chinese chestnut rotten fruit disease
Y. WEI (1), Q. Shang (1), L. Qin (1), S. Liu (1), Z. Liu (1)
(1) Beijing University of Agriculture, Beijing, China
Phytopathology 98:S168

As you well known Chinese chestnut internal rots were a major problem in China. This problem negatively impacted upon consumer confidence and contributed to sell low prices. We examined a lot of samples of chestnut fruits collected from Huairou district, Beijing, China in 2005 and 2006, the average incidence of the disease was about 15% after five months of storage at around 0°C. Moreover, twenty-two different strains of fungi were isolated from the decayed and non-symptom seeds of nut. Sixteen strains were identified to fell into 11 genera, i.e. *Aureobasidium* sp., *Alternaria* sp., *Phoma* sp., *Penicillium* sp., *Trichothecium* sp., *Fusicoccum* sp., *Rhizoctonia* sp., *Mucor* sp., *Colletotrichum* sp., *Botrytis* sp. and *Fusarium* sp. Six strains were not identified yet. Among them, *Alternaria* sp., *Trichothecium* sp., *Fusicoccum* sp. and *Fusarium* sp. had higher isolating rate than others. The inoculation tests showed that *Alternaria* sp., *Fusarium* sp. and *Fusicoccum* sp. could only infect the injured seeds of chestnut, *Trichothecium* sp. could infect both injured and non-injured seeds of chestnut. In order to minimize rots during storage, four preventative treatments before storage were applied. The results showed that the treatments couldn't decrease the rotten rate significantly. Thanks to Academic Human Resources Development in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (PXM2007-014207-044536).

Simple sequence repeats and their potential roles in regulation of contingency genes in phytoplasma genomes
W. WEI (2), X. Shi (2), R. E. Davis (2), D. L. Nuss (1), Y. Zhao (2)
(1) Center for Biosystems Research, University of Maryland Biotechnology Institute, Rockville, MD, USA; (2) Molecular Plant Pathology Laboratory, Agricultural Research Service-USDA, Beltsville, MD, USA
Phytopathology 98:S168

Phytoplasmas are cell wall-less bacteria that parasitize plants and insects. This transkingdom life cycle requires rapid responses to vastly different environments during the sequential host transitions. Features enabling such flexibility in other microbes include simple sequence repeats (SSRs) – mutation-prone, phase variable short DNA tracts that function as "evolutionary rheostats" and enhance rapid adaptations. To gain insights into the occurrence, distribution, and potential functional roles of SSRs in phytoplasmas, we performed computational and experimental analyses on genomes of several '*Candidatus* Phytoplasma asteris'-related strains. The presence of numerous SSRs was first discovered in our computational analyses of the completely sequenced genomes of two phytoplasma strains, aster yellows witches'-broom phytoplasma (AYWB) and onion yellows phytoplasma mild (OYM). Inter-strain diversity of phytoplasma SSRs was remarkable, in terms of both motifs and repeat numbers. Mononucleotide SSRs and trinucleotide SSRs were significantly overrepresented in the AYWB and OYM genomes, when compared with computer-generated random genomes with the same base compositions as actual phytoplasmas. In contrast, higher order SSRs, including dinucleotide SSRs, were underrepresented in the AYWB and OYM genomes. Repeat frequencies of phytoplasmal SSRs decreased exponentially as SSR motif length increased. Findings from computational analyses were complimented by an examination of SSRs in different phytoplasma strains that were maintained in experimental plants. Several SSRs were identified as potential contingency loci due to variations in copy numbers in different strains. Some of these SSRs were located in regions that could profoundly alter the regulation of transcription and translation of affected genes, and/or composition of protein products. The potential impact of these SSRs on associated genes is currently under investigation.

Recurrent phage attacks and subsequent recombination events shaped phytoplasma genome architecture
W. WEI (2), R. E. Davis (2), R. Jomantiene (1), Y. Zhao (2)
(1) Institute of Botany, Vilnius, Lithuania; (2) Molecular Plant Pathology Laboratory, Agricultural Research Service-USDA, Beltsville, MD, USA
Phytopathology 98:S168

Phytoplasmas are cell wall-less bacteria that parasitize plants and insects, causing numerous plant diseases. These transkingdom parasites possess unique genome architecture featured by genes clustered in sequence variable mosaics (SVMs) that arose from recurrent, targeted attacks by unknown mobile elements (Jomantiene and Davis, 2006; Jomantiene *et al*., 2007). In the present study, we discovered a prominent structural component of phytoplasma genomes -- cryptic prophages or phage remnants that may be responsible for SVM formation. We found that, in the genomes of two '*Candidatus* Phytoplasma asteris'-related strains, OYM and AYWB, these prophage remnants comprised 30.5% and 22.6%, respectively, of their circular chromosomes. Differential abundance of prophages accounts for the major part of the overall size difference between the two genomes. These prophage loci occupy nearly all major non-syntenic sections of the AYWB and OYM genomes and cover the entire SVM regions. Originated from integration of tailed double-stranded DNA phages in the family *Siphoviridae*, the prophages have undergone extensive genetic recombination and mutational decay. Clustered phage remnants formed genomic islands that were clearly evidenced by distinct DNA physical signatures such as dinucleotide relative abundance and codon position GC values. Several OYM and AYWB strain-specific genes were identified as phage morons, some of which coincided with hypervariable regions within individual SVMs. Our data strongly suggest that, as platforms for genetic recombination and capture of laterally-transferred genes, prophage remnants continue to play important roles in generating phytoplasma genetic diversity. We further hypothesize that ancient phage attack/integration events led to SVM formation, shaping phytoplasma genome architecture, enabling phytoplasmal transkingdom parasitic lifestyle and pathogenicity, and launching evolution of the phytoplasma clade.

Control of root-knot nematodes by *Bacillus cereus*
L. WEI (1), J. Guo (1)
(1) Plant Protection, Nanjing, Jiangsu, China
Phytopathology 98:S168

Root-knot nematode causes heavy loss of vegetables in China. It is very difficult to control in the field, especially in the plastic house. In this study, *Bacillus cereus* strain AR156 was used to control root-knot nematodes *Meloidogyne* spp. and achieved an good efficacy. Strain AR156 was isolated from Zhongshan Mountain forest soil in Nanjing, Jiangsu province. Its fermenting production caused death of second stage juveniles of *Meloidogyne incognita in vitro* with the mortality of 97%. In greenhouse experiments, biocontrol efficacy of AR156 was around 64%, and average biomass in term of whole plant dry weights increased by 20%. Under field conditions, the average biocontrol efficiency was 74.4% and yield increasement was 179.3%. As for the mechanism of this biocontrol agent, we studied its colonization and other activities. On the 15th day after seed inoculation with 10 ml AR156 suspension in the concentration of 9.63 × 109 cfu/ml, the colonization of this strain was 8.1 × 105 cfu/g in rhizosphere soil of tomato. In addition, this strain could produce some proteinase, cellulose, cellulolytic enzyme and chitinase. It also has the functions of phosphate-dissolving and nitrogen-fixing. In addition, strain AR156 could induce the resistance of tomato against diseases caused by *Ralstonian solanacearum* and *Phytophthora capsici* when we inoculate this bacterium and the pathogens in sepearation way.

Screen of antagonistic fungi for biocontrol of root-knot nematode
B. WEI (1), J. Guo (1)
(1) Nanjing Agricultural University, Plant Pathology Department, Nanjing, Jiangsu, China
Phytopathology 98:S168

We isolated 527 fungal strains obtained from females, eggs and egg masses of the root-knot nematode (RKN) as well as plant roots and rhizosphere soil in Xuzhou, Jiangsu province. The predominant fungal species were *Paecilomyces lilacinus*, *Fusarium* spp., *Pochonia chlamydosporia*, *Penicillium* spp., and *Acremonium* spp. A total of 60 isolates were selected based on strong pathogenicity to RKN, abilities of producing chitinase and proteinase, and were evaluated for their parasitism of eggs, effects on egg hatch and mortality of juvenile. Pathogenicity varied among isolates. Percentages of eggs parasitized were 72.9–96.1% in 4 days after inoculation. Egg hatch rates were 15.7–51.5% compared to that of control group (67.2%), and mortality of juvenile were 49.7–82.5% while that of the control was 12.3% in 7 days after inoculation. Twenty-one fungal isolates with highest pathogenicity were selected to test their biocontrol efficacies in greenhouse experiments. They reduced tomato root gall index by 12.9–46.7%, and biomass increasement were 0.8–22.9%. In field experiments, 5 strains showed biocontrol efficacy to RKN of cucumber from 17.9% to 38.9%, and increased the yield by 2.88–26.39%. Most fascinatingly, control efficacies of 3 strains (*Paecilomyces lilacinus* 518, *Paecilomyces lilacinus* 521 and *Paecilomyces lilacinus* 526) were comparable to avermectin in controlling RKN, while growth promotion were better. All the results suggested that they might serve as eco-friendly economic and easy-to-applied biocontrol agents in the future.

Aggressiveness of *Phytophthora cactorum* and *Phytophthora citricola* isolates on European beech and lilac
J. E. WEILAND (2), A. H. Nelson (1), G. W. Hudler (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA; (2) USDA ARS, Corvallis, OR, USA
Phytopathology 98:S168

Inoculation experiments were conducted to compare the aggressiveness of *Phytophthora cactorum* and *P. citricola* isolates on European beech and lilac

seedlings grown in a greenhouse. The isolates were obtained from bleeding cankers on European beech from 5 cities (Albany, Ithaca, Oyster Bay, Plainview, and Rochester) in New York. Isolates of *P. citricola* were subdivided into 2 clades (*P. citricola* 1 and 2) based on distinct differences within selected DNA sequences. Stems, roots, and leaf disks of both hosts were inoculated with 3 single-spore isolates of *P. cactorum*, 4 of *P. citricola* 1, and 3 of *P. citricola* 2. Stems were inoculated with colonized agar plugs, roots via infested soil at 3 inoculum levels, and leaf disks with a zoospore suspension. Disease incidence was independent of isolate in all inoculated stems and leaf disks (100%), but was dependent on isolate in the soil infestation assay (0–100%) for both hosts. Severity (canker length, rate of mortality, and affected leaf disk area) was dependent on isolate regardless of inoculation site (stem, root, or leaf, respectively) or host, with *P. cactorum* isolates usually causing less necrosis than either clade of *P. citricola*. However, the range of disease severity caused by isolates of *P. citricola* 1 was similar to that of *P. citricola* 2. Lilac was less severely affected by inoculation than beech, regardless of isolate. No effect of inoculum level on root infection was observed.

Identification of NLS signals and a DNA binding domain in the host specificity determinants HsvG and HsvB of the gall-forming *Pantoea agglomerans*

D. M. WEINTHAL (3), S. Manulis-Sasson (1), G. Nissan (3), T. Tzfira (2), G. Sessa (3), S. Barash (3)
(1) A.R.O. The Volcani Center for Agricultural Research; (2) Developmental Biology, The University of Michigan, Ann Arbor, MI, USA; (3) Tel-Aviv University, Tel-Aviv, Israel
Phytopathology 98:S169

Pantoea agglomerans (*Pa*) has been transformed from a commensal bacterium into two related gall-forming pathovars: *Pa* pv. *gypsophilae* (*Pag*) that incites galls on gypsophila and HR on beet and *Pa* pv. *betae* (*Pab*) that causes galls on beet and gypsophila. Pathogenicity of both pathovars was evolved by acquisition of a plasmid-borne pathogenicity island (PAI). Functional type III secretion system (T3SS) and T3SS effectors were mandatory for gall formation. It has been previously reported (Molecular Microbiology 2006, 61:1118-1131) that the effectors HsvG and HsvB are DNA binding proteins localized to host and non-host nuclei and potentially capable of affecting the host transcriptional machinery. HsvG determines host specificity of *Pag* on gypsophila while HsvB determines host specificity of *Pab* on beet. Exchanging the repeats domain between HsvG and HsvB resulted in a switch of host specificities. The current presentation indicates that while no significant similarity to canonical NLS sequences could be revealed in HsvG/B, suspected sites of adjacent arginine and lysine designated as NLS1, NLS2 and NLS3 were detected. By employing pNIA nuclear localization assay in yeast and transient expression of 2xYFP-NLS fusions obtained by particle bombardment of melon leaves, it was demonstrated that either NLS1 or NLS3 but not NLS2 were capable of transporting the 2xYFP into the nuclei and bind AtKAPalpha and importin3alpha of *Arabidopsis*. NLS deletion mutants revealed that NLS1 and NLS3 were necessary for pathogenicity. Deletion analysis of HsvG combined with gel retardation identified the DNA binding domain to five helices in the helix-turn-helix region of HsvG/B.

Genetic characterization of *Acidovorax avenae* subsp. *citrulli* using amplified fragment length polymorphism (AFLP)

A. WEN (2), A. Mangravita-Novo (1), D. L. Hopkins (1), D. J. Norman (1)
(1) Univ. Florida, Mid-Florida REC, 2725 Binion Rd, Apopka, FL 32703-8504; (2) Univ. Florida, North Florida REC, 155 Research Rd, Quincy, FL 32351-5677
Phytopathology 98:S169

Avidovorax avenae subsp. *citrulli* (*Aac*) is the causative agent of watermelon fruit blotch (WFB), a worldwide disease that is particularly destructive in subtropical areas, such as Florida, USA and Queensland, Australia. WFB is currently controlled through the use of clean seed, cultural practices to reduce spread in transplant houses, and copper-containing bactericides. To develop an appropriate disease management strategy, the genetic diversity of the pathogen's populations should be assessed. Until now, the genetic diversity of *Aac* was characterized by pulsed field gel electrophoresis (PFGE) of SpeI-digested DNA and rep-PCR analyses using genomic probes. We used amplified fragment length polymorphism (AFLP) technique with fluorescent labeled primers to identify differences among isolates of *Aac* using ApaI/Taq1 restriction enzyme pair and Apa1 plus G/TaqI plus G as selective primers. Fifty-nine representative strains of this subspecies from international bacterial collections were genotyped. Fifty-eight strains of *Aac* grouped together with 2 subgroups sharing 85% similarities. Strain AAC202-66, an Israeli melon isolate, was an outgroup of the rest of *Aac*, sharing only 56% similarities. *A. facillis*, a non-pathogenic species often isolated from the same eco-system as *Aac*, was easily differentiated from *Aac*, sharing only 47% similarities in their AFLP band pattern. The AFLP band pattern of *A. avenae* subsp. *avenae* is significantly different from Aac, sharing less than 32% similarities.

Control of bacterial spot of tomato with a phosphorous acid product

A. WEN (3), B. Balogh (1), M. Momol (2), S. M. Olson (3), J. B. Jones (4)
(1) Department of Plant Pathology & Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT 06504, USA; (2) District Directors Office, University of Florida/IFAS, Gainesville, FL 32611, USA; (3) North Florida Research and Education Center, University of Florida, Quincy, FL 32351, USA; (4) Plant Pathology Department, University of Florida, Gainesville, FL 32611, USA
Phytopathology 98:S169

K-PHITE, a phosphorous acid-containing product, was evaluated alone or with a combination of other products for management of bacterial spot of tomato incited by *Xanthomonas perforans* in eight greenhouse and seven field experiments in two locations in Florida during a 3-year period. Treatments included (i) a weekly schedule of K-PHITE alone, (ii) K-PHITE combined with full rate or half rate of standard copper-bactericide, (iii) K-PHITE alternated with a standard copper-bactericide, and (iv) K-PHITE plus biweekly Actigard. Both greenhouse and field experiments showed that overall disease control with those treatments was similar to that obtained using the standard copper-bactericide program. Treatment of K-PHITE combined with full rate or half rate of standard copper-bactericide was not superior to the standard copper-bactericide program. Yields in the field experiments were not affected by any of these treatments. Under greenhouse conditions phytotoxicity was observed due to foliar application to tomato seedlings; however, drench application did not cause phytotoxicity. These data suggested that K-PHITE and K-PHITE plus Actigard could be used for managing bacterial spot of tomato in the field in Florida where prevalent natural inoculum is race 3 or 4 of *Xanthomonas perforans*; and that K-PHITE could be used as a new tool in greenhouse transplant production using drench application. A direct effect on bacterial spot of tomato was shown by the greenhouse data with the evidence that K-PHITE acts as antibacterial agent toward *Xanthomonas perforans*, which was confirmed by *in vitro* test. No indirect effect was observed from the greenhouse experiments.

Fighting fungal pathogen by secreting extracellular DNA at pea root tips

F. WEN (1), H. D. VanEtten (1), Z. Xiong (1), M. C. Hawes (1)
(1) Department of Plant Science, University of Arizona, Tucson, AZ, USA
Phytopathology 98:S169

Plant root tips are immune to most soilborne pathogens. The mechanism for this immunity is just starting to be understood. We recently reported that root border cells and their progenitor cells in the root cap synthesize and secrete an array of extracellular proteins. These proteins of the root cap 'secretome' are required to protect the pea root tip from infection by the fungal pathogen *Nectria haematococca*. Here we report that extracellular DNA (exDNA) also plays a key role in root tip resistance to fungal infection. Biochemical and histochemical assays revealed the presence of structural DNA surrounding the pea root tip and border cells. Like the root cap secretome, the exDNA is secreted from living border cells and their progenitor cells during border cell development. Cloning and sequencing analyses show that major components of exDNA are retroelements and repetitive DNA. When the exDNA is destroyed enzymatically, the resistance of the pea root tip to the fungal pathogen *N. haematococca* is abolished, and loss of resistance is correlated with rate of DNA digestion. These data are consistent with the hypothesis that exDNA, together with proteins of the root cap secretome, are components of a plant defense mechanism mediated by structurally integrated extracellular DNA and protein complexes. This previously unrecognized phenomenon in plant cells may reflect a general defense mechanism that is analogous to the mammalian neutrophil extracellular trap (NET), which traps and kills bacteria.

Consequences of tillage intensity on population densities of *Heterodera glcyines* and severity of sudden death syndrome in corn-soybean sequence

A. WESTPHAL (2), H. Mehl (2), A. Seyb (2), T. J. Vyn (1)
(1) Department of Agronomy, Purdue University, West Lafayette, IN 47907; (2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907
Phytopathology 98:S169

Heterodera glycines and sudden death syndrome (SDS) combined cause the most yield losses in U.S. soybean production. In long-term tillage trials with corn-soybean rotation in northern Indiana, more eggs of *H. glycines* were found under high tillage intensity than under no-till averaged for 2006 and 2007, and foliar symptoms of SDS were more severe under high tillage intensity than no-till in 2007. The objective of this research was to investigate the dynamics of *H. glycines* in detail. In spring of 2007, cores of 10-cm diameter and 30 cm depth were collected from both tillage systems (TL),

divided into 10-cm depth layers (DL), and arranged as (AR): (i) non-disturbed; (ii) sieved, original DL maintained; or (iii) sieved, DL reversed. Soybean 'Spencer' was grown in these cores in the greenhouse for two months. At harvest, TL × AR × DL interaction was significant for the reproductive factor (*r*) of the eggs of *H. glycines*. In intensively tilled soil, *r* values were similar for AR, and declined with depth. In the no-till soils, *r* increased slightly with depth in non-disturbed soils; *r* increased more in disturbed soils; and increased strongly in the shallowest but was reduced in the deepest layer in the reversed-soil columns. Soil disturbance had stronger effects on the no-till soil, suggesting that stratified soil factors contribute to differences in population densities between tillage systems. Continuous no-till reduced the incidence of SDS and *H. glycines*.

Mature watermelon vine decline is associated with *Rhizopycnis vagum*
A. WESTPHAL (2), L. Xing (2), S. B. Goodwin (3), D. S. Egel (1)
(1) Department of Botany and Plant Pathology, Purdue University, Vincennes, IN 47591; (2) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907; (3) USDA-ARS, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907
Phytopathology 98:S170

For almost 20 years, mature watermelon vine decline (MWVD) has put sustainable watermelon production at risk in southern Indiana. This late-season vine collapse appears to be specific to watermelon; other cucurbits seem unaffected. To test for a biological cause of MWVD, microplot trials were conducted with two problem soils and various fumigants. The greatest increases in plant growth were observed in plots treated with methyl bromide, the second largest in those with methyl iodide. A 1,3-dichloropropene-chloropicrin treatment had plant growth similar to the non-treated controls. Fungal isolations from root pieces collected from infected watermelon plants at harvest resulted frequently in *Rhizopycnis vagum*, confirmed by sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA. Specific PCR primers designed from the ITS sequence of *R. vagum* consistently amplified the *R. vagum*-specific ITS fragment from infected watermelon roots of plants that had symptoms of MWVD, but not from healthy plants grown in fumigated soil. Pathogenicity tests with *R. vagum* did not replicate MWVD symptoms. The known pathogenicity on cucurbits and the consistent association of *R. vagum* with diseased plants suggests that *R. vagum* plays a role but may not be the sole cause of MWVD. We hypothesize that additional factors are necessary to induce the vine decline.

Genetics of resistance to *Diplocarpon rosae* in tetraploid roses
V. M. WHITAKER (1), S. C. Hokanson (1)
(1) University of Minnesota, Dept. of Horticultural Science, St. Paul, MN, USA
Phytopathology 98:S170

Black spot disease, incited by the fungus *Diplocarpon rosae* Wolf, is the most serious disease of garden roses in the world. Race-specific resistance is well documented in this pathosystem. Two major resistance genes (*Rdr1 and Rdr2*) effective against German races of this pathogen have been introgressed from *Rosa multiflora* into cultivated roses. The objective of this study was to examine the inheritance of resistance to three North American races of *D. rosae* in tetraploid rose cultivars. Cultivars resistant to North American races A, B, and C were crossed to multiple susceptible parents. Progenies were tested by detached leaf inoculation, in which susceptibility was indicated by lesion development and the formation of spore-bearing acervuli. Resistances to races A and C both segregated approximately 1:1 in multiple F_1 progenies, indicative of single dominant genes in simplex (Rrrr) configuration according to random chromosome assortment. In crosses with 'Folksinger' as the female parent, segregation was skewed for resistance to race A, with fewer plants in the resistant class. In crosses between 'Folksinger' and 'Love and Peace', progeny resistant to both races A and C were recovered, demonstrating gene pyramiding as an effective method for gaining broader black spot resistance in rose. Resistance to race B followed a continuous distribution indicative of multigenic control. Resistance was partial, with limited sporulation occurring on the most resistant seedlings. These results will afford valuable information to rose breeders and provide a basis for further genetic studies.

Application of subtractive suppression hybridization in studying differentially expressed genes between pathotypes of *Ascochyta rabiei*
D. White (2), W. CHEN (1)
(1) USDA ARS, Washington State University; (2) Washington State University, Pullman, WA, USA
Phytopathology 98:S170

Ascochyta rabiei causes ascochyta blight of chickpea. Two pathotypes have been identified. The two pathotypes are inter-mating, and no neutral molecular markers were found specific for either pathotype. Here we report using subtractive suppressive hybridization in an attempt to identify pathotype-specific transcripts. Conidia of each pathotype are allowed to germinate on an artificial medium amended with chickpea plant extract. Total RNAs were isolated from the germinating conidia. Representative cDNA pools were generated from the mRNA isolated from each pathotype. A subtractive hybridization procedure was used to eliminate cDNAs that are common to both pathgotypes, to produce enriched populations of cDNA that contain rare and unique transcripts for each pathotype. Differential screening showed that many of the enriched libraries contained transcripts that were common to both pathotypes. However, two unique transcripts were isolated from the pathotype I cDNA library. Two additional transcripts were highly expressed in pathotype I relative to pathotype II. Conversely, four transcripts showed significantly higher levels of expression in pathotype II relative to pathotype I. Sequence analysis of these eight differentially expressed transcripts and homology searches matched gene products ranging from general metabolism to previously identified virulence factors in other fungal species. These sequences provide the information necessary to quantitatively study their differential expression between the two pathotypes.

Population structure of *Colletotrichum* species associated with ripe rot of grapes
K. R. WHITTEN BUXTON (1), T. B. Sutton (1)
(1) North Carolina State University, Raleigh, NC
Phytopathology 98:S170

Ripe rot of grapes, one of the most serious bunch rot diseases in warm humid grape growing regions, is caused by *Colletotrichum acutatum* and *C. gloeosporioides* along with its associated teleomorph *Glomerella cingulata*. To characterize the population structure of this disease complex on grapes, isolates were collected from vineyards of *Vitis vinifera* and *Muscadina rotundifolia* as well as fruit plantings and wild *Vitis* spp. adjacent to the vineyards from 2004–2007. Colony growth and spore morphology were used for initial isolate identification, which was later confirmed using species specific PCR primers CaInt2 and CgInt. Nitrate nonutilizing (nit) mutants were then generated, characterized, and paired to determine vegetative compatibility groupings (VCG). Three genes (gene 1, 2, 3) were sequenced from a small subset of each of the VCGs; genealogies were inferred separately for each gene and examined for concordance.

Biology and epidemiology of *Colletotrichum* species associated with ripe rot of grapes
K. R. WHITTEN BUXTON (1), T. B. Sutton (1)
(1) North Carolina State University, Raleigh, NC, USA
Phytopathology 98:S170

Colletotrichum spp. and the associated teleomorph *Glomerella* are cosmopolitan pathogens which cause losses on many different crops; however, little is known of the biology and epidemiology of these pathogens on grape. Ripe rot of grape is caused by *C. gloeosporioides*, *C. acutatum*, and *G. cingulata* and is one of the most important bunch rots in warm, humid grape growing regions. To determine the susceptibility of fruit through the growing season, biweekly inoculation of cultivars Cabernet Franc, Chardonnay, and Seyval blanc were conducted from bloom to preharvest from 2005 to 2007. While grapes of all cultivars were susceptible to infection throughout the growing season, fruit of Seyval blanc and Chardonnay were most susceptible during bloom and veraison while Cabernet Franc was most susceptible at closing and preharvest. To determine susceptibility of commonly grown grape cultivars, 35 cultivars of *Vitis vinifera*, *Muscadina rotundifolia*, *V. aestivalis*, *V. labruscana*, and French American hybrids were inoculated with *G. cingulata* from 2005 to 2007. Most resistant cultivars included Merlot, Cynthiana, and Pride, while highly susceptible cultivars included Cabernet Sauvignon, Carlos, and Seyval blanc.

Developing and validating a greenhouse bioassay for Potato tuber necrotic ringspot disease (PTNRD) associated with Potato virus Y
J. L. WHITWORTH (2), S. M. Gray (3), A. Karasev (4), J. H. Lorenzen (1)
(1) CGIAR, Kampala, Uganda; (2) USDA-ARS, Aberdeen, ID, USA; (3) USDA-ARS, Ithaca, NY, USA; (4) University of Idaho, Moscow, ID, USA
Phytopathology 98:S170

The recent discovery of necrotic strains of Potato virus Y (PVY) in the U.S. has prompted a need to determine the susceptibility of the commonly grown potato cultivars to potato tuber necrotic ringspot disease (PTNRD). PVY strains are commonly differentiated by serological and molecular assays; however they do not always predict the ability of a PVY isolate to cause PTNRD, necessitating the need for bioassays. Positive bioassay results are characterized by veinal necrosis in tobacco and tuber necrosis in potato. PTNRD is obvious in field grown tubers; however, development of a greenhouse based bioassay on minitubers would reduce the length of the bioassay and allow year round testing in controlled conditions that could be started at any time. A bioassay was developed which included a spray gun system to allow inoculation of a large numbers of potato or tobacco plants. We compared the incidence and expression of PTNRD in mini-tubers grown

in the greenhouse and tubers grown in the field. Foliar symptoms were recorded weekly and PTNRD symptoms were recorded at harvest and at least four weeks post-harvest. Over two years, Yukon Gold, Ranger Russet and A93157-6LS cultivars were inoculated and evaluated in the greenhouse and field with 5 PVY isolates representing O, NTN, and N:O strains. For the purpose of evaluating the ability of a PVY isolate to cause PTRND, Yukon Gold gave the most consistent bioassay results in the field and greenhouse both years. For evaluating other cultivar/clone reactions in the greenhouse, it may be necessary to replicate the bioassay over time.

NIR spectroscopy as a tool for optimizing sorting of white maize kernels contaminated with mycotoxins

D. T. WICKLOW (2), T. C. Pearson (1), D. Brabec (1)
(1) USDA-ARS-GMPRC, Manhattan, KS, USA; (2) USDA-ARS-NCAUR, Peoria, IL, USA
Phytopathology 98:S171

Maize kernels highly contaminated by aflatoxin and fumonisin are unevenly distributed in a grain lot and may be concentrated in a very small percentage of the product. Near-infrared (NIR) reflectance spectra (500-1,700 nm) were analyzed to select the pair of absorbance bands (filters) giving the lowest classification error rate for removing whole white maize kernels contaminated with aflatoxin or fumonisin in a single pass through a commercial high speed sorter (@7,000 kg/hr). It was found that using the wavelength pair of 500 nm and 1200 nm, approximately 87% and 93% of kernels having high levels of aflatoxin (>100 ppb) and high levels of fumonisin (>40 ppm), respectively, were correctly classified. Additionally, approximately 96% of the kernels having low levels of aflatoxin (<10 ppb) and fumonisn (<2 ppm) were correctly classified as uncontaminated. Kernels having minor symptoms (25% to 50% discolorations) had lower classification accuracies (80%), than those with discolorations covering more than 75% of the kernel (88%), or discolored BGYF kernels (91%). A commercial sorting machine (Satake DE) was set up to sort corn using light at 500 and 1200 nm and reject 4% to 9% of the incoming corn in three lots ranging from 23 to 150 ppb aflatoxin and 0.4 to 0.6 ppm fumonisin. Because some kernels with minor symptoms of discoloration had very high contamination levels of aflatoxin, two passes through the sorter were required to reduce aflatoxin below the regulatory limit of 20 ppb. In earlier sorting experiments with commercially harvested yellow corn contaminated with equivalent levels of aflatoxin (average 75 ppb), we were able to reduce aflatoxin below 20 ppb with a single pass through the same Satake DE machine. Differences in pericarp thickness, kernel vitreosity and the presence or absence of carotenoids, could influence both kernel symptom expression and the ability of NIR light to detect fungal damaged endosperm.

Evaluation of intraspecific competition (*Aspergillus flavus* Link) and aflatoxin formation in suspended disc culture and preharvest maize

D. T. WICKLOW (1)
(1) USDA-ARS-NCAUR, Peoria, IL, USA
Phytopathology 98:S171

The abilities of non-aflatoxin producing strains of *Aspergillus flavus* NRRL 32354; 18543; 21882; 21368 as well as domesticated koji strains *Aspergillus oryzae* (syn. *A. flavus* var. *oryzae*) NRRL 451; 1911; 5592; 6271; 30038 to interfere with aflatoxin formation by A. flavus NRRL 3357; 32355 were examined in suspended disc culture (SDC) and by wound-inoculating maize ears in the late milk to early dough stage of maturity at Kilbourne, Illinois in 2001 and 2002. In SDC aflatoxin yields were substantially reduced when conidia were mixed in equal proportions at a constant total density 1×10^5 spores/ml^{-1}. Non-aflatoxin producing strains of *A. flavus* reduced aflatoxin yields in NRRL 3357 by an average of 70% and NRRL 32355 by an average of 95%, while strains of *A. oryzae* reduced aflatoxin yields by an average of 62% & 73%. In pre-harvest maize (2001 & 2002) non-aflatoxin producing strains of *A. flavus* reduced aflatoxin levels in NRRL 3357 by an average of 63% & 79% but were largely ineffective in suppressing aflatoxin levels when paired with NRRL 32355 (red. 26% & incr. 41%). The parasitic ability of competing strains may be as important as intraspecific competition in determining the extent to which the seed becomes contaminated with aflatoxin. In both years, non-aflatoxin producing strains of *A. oryzae* supported substantially greater aflatoxin yields when paired with NRRL 32355 (incr. 70% & incr. 377%), which may be the result of nutritional cross-feeding. *Aspergillus flavus* populations in the central corn belt may include a majority of strains that produce no aflatoxin and thus could function naturally in suppressing the severity of aflatoxin outbreaks.

Comparing New Zealand and United Kingdom isolates of *Phytophthora kernoviae*

T. L. WIDMER (1)
(1) USDA, ARS, FDWSRU, Fort Detrick, MD
Phytopathology 98:S171

Phytophthora kernoviae was discovered in the United Kingdom in 2003 and identified as a new species in 2005. Recent DNA sequence studies identified two unknown *Phytophthora* isolates collected in the 1950s and 2002 in New Zealand as *P. kernoviae*. The purpose of this study was to compare two isolates originating from New Zealand (PK-1 and PK-2) and two isolates originating from Cornwall, UK (PK-3 and PK-4). Mycelial growth on agar plates was similar for all isolates at 5, 10, 15, 20, 25, and 30°C. Sporangial production was about five to 10 times higher in liquid cultures for PK-3 and PK-4 than PK-1 and PK-2. However, isolate PK-3 could not be induced to release zoospores. *P. kernoviae* is homothallic and easily produces oospores in culture. Oospore production was similar for PK-1, PK-2 and PK-4, which were about three times higher than for PK-3. Inoculation of Rhododendron leaf disks with sporangial or oospore suspensions showed little difference in necrosis among the isolates, except for PK-3, which showed very little necrosis. Whole plant inoculations of *Magnolia stellata* and *Rhododendron* "Cunningham's White" showed higher necrosis when inoculated with sporangia of PK-1 and PK-2. These results show differences between the New Zealand and UK isolates of *P. kernoviae*. Future tests should include at least one isolate from each geographic location.

Use of a GUS reporter system to characterize the regulon controlling syringomycin production in *Pseudomonas syringae* pv. *syringae*

J. L. WILLIAMS (1), D. C. Gross (1)
(1) Texas A&M University, Department of Plant Pathology and Microbiology, College Station, TX
Phytopathology 98:S171

The non-ribosomally synthesized phytotoxins, syringomycin and syringopeptin, serve as important virulence determinants of *Pseudomonas syringae* pv. *syringae* (*P.s.s.*). These pore-forming, cyclic lipopeptides are associated with the formation of necrotic leaf spot symptoms on susceptible host plants. Thus far it is known that toxin production is activated by bacterial detection of plant signal molecules. Additionally the global regulator and two-component signal transduction system, GacA/GacS, is known to serve as a major component in the regulon controlling syringomycin (*syr*) production by activating the LuxR-like protein, SalA. SalA positively regulates another LuxR-like protein, SyrF, which in conjunction with SalA positively regulates the expression of the *syr* synthetase gene, *syrB1*. Although the genetic organization and biosynthetic mechanism for syringomycin has been studied in detail, the regulatory network required for activation of the 42-kb syr gene cluster remains incomplete. Random mutagenesis of a *P.s.s.* B301D *syrB1::uidA* reporter construct (B301DSL8), using the EZ-Tn5™ transposon insertion method, was used to analyze and identify additional members of the *syr* regulatory network. By utilizing this beta-glucuronidase (GUS) reporter system, a simple and reproducible screening process was developed to detect the loss of *syrB1* expression via a lack of fluorescent color change. The development of this random mutagenesis and screening process expands our current knowledge of the regulatory network controlling syringomycin biosynthesis by identifying new components of the phytotoxin regulon.

Strand-specific real-time RT-PCR quantitation of Maize fine streak virus genomic and positive-sense RNAs using high temperature reverse transcription

D. K. WILLIS (2), T. L. German (1)
(1) Department of Entomology, UW-Madison, Madison, WI, USA; (2) USDA-ARS Vgetable Crops Reserach Unit, Department of Plant Pathology, UW-Madison, Madison, WI, USA
Phytopathology 98:S171

Efforts to analyze the replicative RNA produced by *Maize fine streak virus* (MFSV) within maize tissue was complicated by the lack of specificity during cDNA generation using standard reverse transcriptase protocols. Real-time qRT-PCR using cDNA generated by priming with random hexamers does not distinguish between virion (vRNA) and virion-complementary (vcRNA) or viral mRNA. Detection and quantitation of the products of viral replication requires strand-specific cDNA synthesis. However, auto-priming (generation of cDNA without primers) of the vRNA or vcRNA and false priming of the incorrect strand complicate detection and quantitation of viral replicative RNAs. Our work describes the identification of efficient primers specific to each of the seven MFSV ORFs as well as vRNA and vcRNA. Strand-specificity was improved by increasing cDNA reaction temperature from 42°C to 60°C with tagged primers which reduced auto-priming 23-fold and non-specific priming by 21 to 315-fold. Using this methodology, we established that MFSV vRNA is 30 to 60-fold more abundant than the replicative vcRNA in maize leaf tissue exhibiting fine streak symptoms. In contrast to mRNA ratios established in the well studied rhabdovirus *Vesticular stomatitis virus*, the N, P and L gene messages of MFSV were statistically equivalent in symptomatic maize leaf tissue.

Detection of trichothecene mycotoxins and ergosterol within wheat florets using gas chromatography with electron capture detection

K. T. WILLYERD (2), D. D. Archibald (2), K. Boroczky (2), E. D. De Wolf (1), G. A. Kuldau (2)
(1) Kansas State, Manhattan, KS, USA; (2) Penn State, University Park, PA, USA
Phytopathology 98:S172

The fungus *Fusarium graminearum* is the primary causal agent of head blight of wheat in the United States. In addition to causing yield reductions, *F. graminearum* also contaminates grain with trichothecene mycotoxins, most notably deoxynivalenol (DON). However, the relationship between mycotoxin and fungal levels within wheat heads is not fully understood, in light of the fact that DON can be translocated through the plant. The objective of this study was to develop a method to detect and quantify trichothecenes and ergosterol, a sterol unique to fungal cell membranes, in small amounts of wheat tissue, such as single florets. Gas chromatography (GC) with electron capture detection was chosen for this analytical method due to its sensitivity. Trichothecenes, including DON and acetylated derivatives, were extracted from wheat florets with 84:16 acetonitrile-water. Ergosterol was subsequently extracted from the same florets with hexane after saponification with methanolic potassium-hydroxide. Extracts were derivatized with hepta-fluorobutyric anhydride and a single GC oven program was used to detect trichothecenes and ergosterol. The detection limit for DON was 10 pg/µL and 500 pg/µL for ergosterol, levels of sensitivity adequate to detect these compounds in single wheat florets. This method was employed to examine infection and trichothecene accumulation patterns within wheat heads. Following the inoculation of a central spikelet with *F. graminearum* macroconidia, wheat plants were incubated at either 15 or 22°C. Single wheat florets were harvested from inoculated heads every other day for 10 days. Results regarding disease incidence, fungal growth and toxin translocation will be discussed.

Incidence of bacterial wetwood in southern bottomland hardwood logs and lumber

A. D. WILSON (1)
(1) USDA Forest Service, Southern Hardwoods Lab, Stoneville, MS, USA
Phytopathology 98:S172

Bacterial wetwood is a major problem affecting hardwood lumber production in the United States. This disease causes high economic losses to the forest products industry in many ways. The problem is particularly prominent in southern bottomland hardwood forest stands that sustain prolonged periods of flooding. The primary objective of this study was to determine the magnitude of this problem to the local lumber industry in the central delta region of the Mississippi Alluvial Valley. A survey of wetwood damage to raw logs of twelve tree species, examined at a hardwood lumber mill in Vicksburg, MS, indicated very high incidence of the disease (>95%) in cottonwood (*Populus deltoides*); intermediate incidence (39%) in black cherry (*Prunus serotina*), 31% in bald cypress (*Taxodium distichum*), 31% in basswood (*Tilia americana*), 19% in certain red oaks (e.g., *Quercus falcata*); low incidence (7%) in green ash (*Fraxinus pennsylvanica*), and 2% in sugarberry (*Celtis laevigata*). The type and severity of wetwood damage varied considerably for each tree species. Processed and kiln-dried lumber derived from wetwood logs exhibited a diversity of damage types and patterns for different lumber types. Lumber degrade consisted primarily of various cracks (checking), distortions, and discoloration (brown stain) damage. These results will be useful for constructing wetwood hazard ratings for individual tree species in this region.

Long-term effect of a single application of factory waste lime on sugar beet and Aphanomyces root rot

C. E. WINDELS (2), J. R. Brantner (2), A. L. Sims (2), C. A. Bradley (1)
(1) University of Illinois, Crop Sciences Dept., Urbana, IL, USA; (2) University of Minnesota, NW Res & Outreach Ctr., Crookston, MN, USA
Phytopathology 98:S172

Aphanomyces cochlioides infests over 50% of sugar beet fields in Minnesota (MN) and North Dakota (ND). Calcium carbonate aids extraction of sucrose from beet juice by precipitating impurities; by-product "waste lime" contains inorganic and organic compounds. Long-term effects of one soil-application of waste lime on sugar beet yield and Aphanomyces root rot was studied by amending plots with 0, 6, 12, 18 and 24 Mg dry wt per ha at Breckenridge (MN) and 0, 7, 15, 29 and 44 Mg dry wt per ha at Hillsboro (ND). In 2007, sugar beet was sown for the first time, 37 and 43 months after lime was applied at MN and ND, respectively. At MN, root rot was severe and interactions occurred between lime rate and cultivar (susceptible [S] and moderately resistant [MR] + hymexazol). The S cultivar had more root rot and lower yield compared to the MR cultivar in the non-limed control; for both cultivars, root rot decreased and yield increased with increasing rates of lime. At ND, soil was too dry for root rot and S and MR cultivars responded similarly; there was a trend for higher stands and yields with increasing rates of lime compared to the control. Thus, a soil amendment of waste lime that was applied over 36 months earlier, decreased Aphanomyces root rot and increased sugar beet yield (especially when *A. cochlioides* was active) compared to a non-limed control.

Differential interference with fatty acid degradation and *Pythium ultimum* sporangium activation by seed exudate sugars explains biocontrol failure of *Enterobacter cloacae*

S. Windstam (1), E. B. NELSON (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA
Phytopathology 98:S172

Enterobacter cloacae protects plants such as cucumber but not others such as corn from seed infections by *Pythium ultimum*. One of the most likely explanations for the failure of *E. cloacae* to control *P. ultimum* on corn is that the bacterium fails to degrade sporangium-stimulating long chain unsaturated fatty acids (LCUFA) in the spermosphere. To test this, our studies focused on the early sensing and response behavior of *P. ultimum* sporangia to seed exudates along with the exudation of fatty acids and sugars from seeds in the presence or absence of *E. cloacae*. Nearly complete activation of sporangia was observed in the corn spermosphere within 30 min of sowing and only low levels of activation in the cucumber spermosphere. Saturated and LCUFA were readily detected in corn and cucumber spermospheres as early as 15 min after sowing. LCUFA from both plant species consisted almost exclusively of oleic and linoleic acids. Fatty acid degradation mutants of *E. cloacae* failed to reduce sporangial activation in the cucumber or corn spermospheres. *E. cloacae* degraded linoleic acid at rates of 29-39 ng/min, exceeding the rate of total fatty acid from seeds. Yet in the presence of exudate sugars, linoleic acid degradation by *E. cloacae* decreased to 7.5-8.8 ng/min, leaving sufficient concentrations of linoleic acid to stimulate the germination of *P. ultimum*. Corn seeds released concentrations of sugars sufficient to represses LCUFA degradation into the spermosphere within 30 min of sowing. Cucumber seeds released only very low levels of sugars. Our results indicate that the failure of *E. cloacae* to protect corn is due to the presence of high exudate sugars that repress fatty acid degradation, ultimately allowing the activation of sporangia in the spermosphere.

Resistance to curly top viruses through virus induced gene silencing

W. M. WINTERMANTEL (1), L. L. Hladky (1)
(1) USDA-ARS, Salinas, CA, USA
Phytopathology 98:S172

Curly top disease, caused by viruses of the genus Curtovirus, and transmitted by the beet leafhopper (*Circulifer tenellus*), has resulted in losses for western U.S. agriculture for over a century. No control methods have been developed that economically, effectively and reliably prevent losses in tomato, sugarbeet and many other crops, and sources of host resistance are incomplete and difficult to transfer among cultivars. In order to provide more reliable control in a wider array of hosts, we are developing methods to engender resistance to the two primary curtovirus species in the United States. Partial replication gene (C1) sequences of *Beet severe curly top virus* (BSCTV) and *Beet mild curly top virus* (BMCTV) were inserted into a *Tobacco rattle virus* (TRV)-based vector to test the effectiveness of the sequences in suppressing infection of BSCTV and BMCTV through virus-induced gene silencing (VIGS). TRV containing curtovirus VIGS-inducer constructs were agroinoculated into *Nicotiana benthamiana* seedlings. BSCTV and BMCTV were inoculated separately at various time points following treatment with TRV/VIGS inducers. Test plants were monitored for the development of curly top symptoms over time and scored for disease severity, plant weight and virus concentration. Results with two silencing constructs delayed and reduced curly top symptom development in infected plants and decreased virus concentration compared to plants not treated with silencing constructs. Constructs that performed well using the VIGS system are being transformed into *N. benthamiana*.

Whole-tree water relations of western gall rust infected lodgepole pine trees in response to soil drought

J. M. WOLKEN (1), P. V. Blenis (1)
(1) University of Alberta
Phytopathology 98:S172

One-year-old lodgepole pine [*Pinus contorta* Dougl. ex Loud.] trees were either inoculated with western gall rust [WGR; *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka] or left as controls. Drought stress was applied one year later under either greenhouse (GH) or growth chamber (GC) conditions. The 4-hr whole-tree transpiration (E; g H2O), soil water content (WC; %H2O (v/v)), and leaf and soil water potentials (MPa; psi_{leaf} and psi_{soil}, respectively) were measured daily. The difference in water potential between the soil and the leaves (Delta psi; MPa), and the whole-tree hydraulic conductivity (K_{psi} ; g

H_2O cm^{-1} MPa^{-1}) and hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}) were calculated. The E of galled and control trees in the GH and GC was constant until the soil water content reached 11%, after which E decreased linearly with decreasing soil WC. The data were analyzed separately for well-watered (soil WC $\geq$ 11%) and water-limited (soil WC $\leq$ 8%) conditions. Analysis of variance was performed on the natural log transformed (ln) variables comprising K_{psi} and Q_L, to partition $\ln K_{psi}$ and $\ln Q_L$ into $\ln K_{psi} = \ln E + \ln L - \ln A_S -$ lnDelta psi, and $\ln Q_L = \ln E + \ln L - \ln A_L -$ lnDelta psi, respectively (A_S and A_L are the sapwood area and leaf area, respectively). Under well-watered conditions, galled trees had reduced A_L relative to control trees, likely because of reduced K_{psi}. However, the reduction in A_L of galled trees did not compensate for their lower K_{psi} values, and thus galled trees had lower Q_L and greater Delta psi than control trees. Under water-limited conditions galled and control trees responded similarly. The similar short term behavior of galled and control trees to drought stress suggests that gall-induced mortality results from indirect long-term growth reductions rather than from runaway xylem embolism.

Surveys of wheat viruses in the Texas Panhandle

F. WORKNEH (1), D. C. Jones (1), G. B. Heidel (1), J. A. Price (1), C. M. Rush (1)
(1) Texas AgriLife Research, Amarillo
Phytopathology 98:S173

The Texas Panhandle produces about half of the state's annual hard red winter wheat crop and diseases caused by viruses are some of the major production problems in the region. Wheat production in this region is dual purpose (grain and grazing) and virus diseases impact both production categories. Routinely encountered viruses are Wheat Streak Mosaic Virus (WSMV), Wheat Mosaic Virus (WMoV) and Barley Yellow Dwarf Virus (BYDV). WSMV and WMoV are vectored by the wheat curl mite, Aceria tosichella Keifer, while BYDV is vectored by several species of aphids. To determine the relative incidences of these viruses, surveys were conducted in which samples were collected from 167, 72, and 33 fields in 2000, 2001, and 2002, respectively. County agents assisted in the survey by collecting 5 foliage samples from each field and submitting them to the diagnostic laboratory in Amarillo for processing. In 2006 and 2007, one sample per field was arbitrarily collected from 112 and 33 fields, respectively. In addition, in 2006, two fields were grid sampled each containing 60 sampling locations. The survey samples were collected in the spring of each year while the grid samples were collected from each field both in the fall and the spring. The samples were tested for the viruses using ELISA. In all survey samples, WSMV was the most dominant virus ranging from 6% of the samples testing positive in 2007 to 93% in 2006 followed by BYDV which ranged from 0 in 2007 to 40% in 2000. Overall, WMoV was the least encountered virus with incidences ranging from 0% in 2007 to 31% in 2006. WSMV also was the predominant virus in grid-sampled fields.

Sudden aspen decline in southwest Colorado

J. J. WORRALL (1), R. A. Mask (1), T. Eager (1), L. Egeland (1), W. D. Shepperd (2)
(1) USDA Forest Service, Gunnison, CO, USA; (2) USDA Forest Service, Rocky Mountain Research Station (retired), Fort Collins, CO, USA
Phytopathology 98:S173

Sudden aspen decline (SAD) has increased rapidly in recent years, approaching 350,000 acres in Colorado in 2007, or 13% of the aspen cover type. We investigated the severity, site/stand factors and causes associated with SAD in southwest Colorado. First, we documented landscape (GIS-DEM analyses) and stand factors (stand exams). There was a strong inverse relationship between elevation and damage, damage tended to occur on south and southwest aspects, was most severe in open stands with large trees, and regeneration was poor in damaged stands. Five biotic agents were frequently associated with mortality: Cytospora canker, poplar borer, bronze poplar borer, and two bark beetle. We proposed a causal hypothesis in a decline context: *Predisposing factors*: low elevations, south/west aspects, low density, stand maturity; *Inciting factors*: warm drought conditions; *Contributing factors*: secondary, biotic agents mentioned above. The second phase is a field survey with 76 plots on four National Forests. Analyses indicate: (a) Regeneration has not responded significantly to crown loss; (b) Root mortality varied from 0 to over 90% of root volume and was correlated with crown loss, and damaged plots had significantly higher volume of dead roots than healthy plots; (c) Regeneration decreased significantly as root mortality increased in damaged plots, but not in healthy plots; (d) Crown loss did not vary significantly with depth of soil mollic layer; (e) Crown loss did not vary significantly with average or oldest age of sampled codominant/dominant trees. There are significant management implications and may be loss of aspen cover type where aspen stands are declining and regeneration is inadequate. Marginal regeneration may be further compromised by such factors as amount and duration of ungulate browsing.

Post-fumigation horizontal and vertical recolonization of soil by *Verticilliium dahliae*

B. M. WU (1), S. T. Koike (1), G. E. Vallad (2), K. V. Subbarao (1)
(1) UC Davis, Salinas CA; (2) University of Florida, Wimauma, FL
Phytopathology 98:S173

A lettuce field infested by *Verticillium dahliae* was fumigated in 2002 fall, and then planted with various crops in 4 different blocks from 2002 to 2006. Soil samples were collected in spring in the 4 blocks in 5-cm increments to a depth of 60-cm during 2003–2006 to determine vertical recolonization by *V. dahliae*. In two other infested fields, soil samples were collected at three sites each in an 8×8 grid (64 1×1-m quadrats) in 2004 before fumigation, and post–fumigation during 2005 through 2007 to determine the horizontal recolonization by *V. dahliae*. During both studies, strawberry was planted post-fumigation followed by various leafy vegetables or crucifers. All soil samples were assayed for *V. dahliae* microsclerotia in laboratory. Results demonstrated that some microsclerotia remained viable after fumigation, fumigation offered short term disease suppression, and recolonization of soil by *V. dahliae* was highly dependent on the subsequent crops. Density of microsclerotia declined after crops such as strawberry, radicchio, and cabbage, but increased after lettuce crops. Microsclerotium densities were greater at 5 to 15-cm depths than at greater depths. During non-host crops, density of microsclerotia declined early, more rapidly near the soil surface than at greater depths, and stabilized later. In the second study, the density of microsclerotia before fumigation followed the same patterns of disease incidence, high near the edges and progressively decreased inwards, suggesting the introduction of inoculum via contaminated equipment and personnel. Post-fumigation recolonization was greatest in areas that had the highest concentration of microsclerotia prior to fumigation.

Genetic structure of North American populations of *Phoma sclerotioides*, causal agent of brown root rot of alfalfa

M. J. WUNSCH (1), G. C. Bergstrom (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA
Phytopathology 98:S173

Phoma sclerotioides, causal agent of brown root rot of alfalfa, is associated with winterkill and poor spring regrowth of alfalfa and other perennial forage legumes in regions with harsh winters. Single-conidium isolates of the fungus were obtained from diseased alfalfa roots and crowns collected in western, central and eastern United States in 2004 to 2007 and central and eastern Canada in 2007. Diagnostic PCR of the isolates using previously developed *P. sclerotioides*-specific primers resulted in a single amplicon of expected size. On potato dextrose agar (PDA) and on water agar with barley, all isolates produced large pycnidia with multiple beaks, white cirri darkening to yellow with age, and unicellular, hyaline, ovoid conidia. Isolates differed in mycelial pigmentation and in production of aerial mycelium on PDA, with two or more culture types represented in each region. The internal transcribed spacer (ITS) 1, 5.8S, and ITS2 of the rDNA and intron-spanning regions coding for actin, beta tubulin, and glyceraldehyde 3-phosphate dehydrogenase were sequenced and analyzed. The multilocus sequence typing (MLST) data indicate that the isolates identified as *P. sclerotioides* are diverse, with isolates falling into two or more distinct clades in each region sampled. Clades correspond to culture types on PDA. Phylogenetic analysis of the MLST data and relative pathogenicity of representative isolates from the different clades will be presented.

Developing a fungicide resistance management guide for vegetable crops grown in the mid-Atlantic region

C. A. WYENANDT (4), K. L. Everts (3), R. L. Mulrooney (1), S. L. Rideout (2), N. L. Maxwell (4)
(1) Dept. of Plant & Soil Sciences, University of Delaware, Newark, DE 19716; (2) Dept. of Plant Pathology, Physiology and Weed Sciences, Virginia Polytechnic Inst., ESAREC, Painter, VA 23420; (3) Dept. of Plant Sciences & Landscape Architecture, University of Maryland, Lower Eastern Shore Res. & Ed. Center, Salisbury, MD 21801; (4) Rutgers University, New Jersey Agricultural Experiment Station, Bridgeton, NJ 08302
Phytopathology 98:S173

In the mid-Atlantic region (NJ, MD, VA, DE, PA) of the United States approximately 90,000 ha of fresh-market and processing vegetable crops are grown each year. Over the past decade, new fungicide chemistries with specific modes-of-action have been developed for use in commercial vegetable production. Many of these new fungicides have a high-risk for fungicide resistance development. The number of fungicide chemistries available and differences in modes-of-action can make it very difficult for vegetable growers to develop and follow fungicide resistance management programs. In 2007 and 2008, using Fungicide Resistance Action Committee (FRAC) codes, we developed a fungicide resistance management guide with tables for the 30 crop groups listed in the mid-Atlantic Commercial Vegetable Production Recommendation Guide. Each FRAC table consists of all

fungicide recommendations for a crop (or crop group) along with FRAC and risk management codes, diseases for that crop (or crop group) and fungicide resistance management guidelines for each particular FRAC code. This simple-to-use reference guide for keeping track of fungicide use was developed to help vegetable growers i) understand the importance of knowing FRAC codes, ii) determine proper fungicide chemistry rotations, and iii) help reduce the potential for fungicide resistance development. In 2007, 560 guides were distributed to vegetable growers representing approximately 17,000 ha of vegetable production in the mid-Atlantic region.

Genome-wide pyrosequencing analysis of a *Citrus tristeza virus* (CTV) complex revealed large-scale recombination throughout the viral genome

Z. XIONG (2), Z. Weng (2), Y. Yu (2), S. Gowda (1), X. Liu (2), D. W. Galbraith (2), R. A. Wing (2), W. O. Dawson (1)
(1) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA; (2) Department of Plant Sciences, University of Arizona, Tucson, AZ, USA
Phytopathology 98:S174

Recombination is a major force driving virus evolution, especially in viruses that have high genome stability and persistently infect hosts with multiple strains. CTV, an RNA virus with a genome of approximately 20 kb, occurs frequently as a complex of multiple strains in its natural hosts, vegetatively propagated and long-lived citrus plants. To examine the CTV complex at the sequence level and to assess the extent of inter-strain recombination, we performed deep-sequencing analysis of a natural CTV complex, FS2-2, using the high-throughput, 454 pyrosequencing technique. Entire CTV genomes in FS2-2 were amplified with four sets of universal RT-PCR primers and subjected to the 454 sequencing analysis in a 1/16-region sequencing run in the Genome Sequencer FLX. Over 2.2 megabases of high quality sequences were obtained from 8722 sequencing reads with an average read length of 256 nucleotides. Three divergent, high coverage (27X-43X) genome contigs corresponding to the genomes of the three co-infecting CTV strains were subsequently assembled from the sequence reads. Additionally, a large percentage (4.7%) of the sequencing reads represented recombinants between the three strains. A genome-wide recombination map for each of the CTV strains in FS2-2 was constructed using these recombinant sequences, revealing a systematic and unprecedented scale of recombination activity throughout the CTV genome. Recombination was more active in the more highly conserved 3′ halves of the CTV genomes. This unprecedented, genome-wide recombination provides a plausible explanation for rapid evolution and extreme diversity of an RNA virus whose genome is remarkably stable for years in hosts infected with a single pure strain.

New strategy to enhance rice resistance to fungal pathogens

T. XU (2), M. Liu (2), Z. Sun (4), G. E. Harman (1), M. Lorito (3), S. L. Woo (3)
(1) Department of Horticultural Sciences, Cornell University, Geneva, NY, USA; (2) Department of Plant Protection, Zhejiang University, Zhejiang, China; (3) Institute of Plant Pathology, Napoli University, Tritici, Italy; (4) Key Laboratory for Rice Biology, Ministry of Agriculture, China National Rice Research Institute, Hangzhou 310006
Phytopathology 98:S174

Rice sheath blight (*Rhizoctonia solani*) and blast (*Magnaporthe grisea*) are two serious diseases in China. Resistance breeding is a key strategy to control the diseases. However, rice cultivars with broad spectrum, high level and durable resistance to the pathogens have been difficult to obtain by traditional breeding due to the lack of resistant germplasm to *R. solani* and the high genetic diversity of *M. grisea*. Trichoderma strains were demonstrated to encode proteins with high antifungal activity against a wide range of plant pathogenic fungi. The potential of the biocontrol agent Trichoderma strains and their genes encoding for fungal cell wall degrading enzymes (CWDE) to enhance rice resistance against the pathogens was investigated in greenhouse. The disease severities of blast and sheath blight on rice cultivar Yuanfengzao pretreated by seed coating with T. harzianum NF9 were significantly reduced and the rice seedlings exhibited a moderate resistance to both of the pathogens. Agrobacterium mediated transformation of rice with three genes encoding for CWDE, ech42 (endochitinase), nag70 (exochitinase) and gluc78 (exo-1,3-beta-glucanase) from *Trichoderma atroviride* was conducted with transformation rates ranging from 24.6 to 55.5%. A total of more than 1,800 independently regenerated plantlets in seven different populations were obtained. The results from Southern blot analysis of the PCR-positive plants further confirmed that the exogenous genes were inserted into rice genome. Enhanced resistance to blast was found in all types of regenerated plants, while a few lines expressing ech42 appeared to be immune to this pathogen. The expression of the ech42 gene increased resistance to sheath blight and the expression level was correlated with disease resistance. In addition, exochitinase enhanced the positive effect of endochitinase on disease resistance when two genes were co-expressed in transgenic rice. This work was supported by the Sino-Italy Joint Research Project (2006DFA32900).

Development of a rapid method to screen hosta cultivars for resistance to hosta petiole rot caused by *Sclerotium rolfsii* var. *delphinii* based on application of oxalic acid

Z. XU (1), M. L. Gleason (1), D. Mueller (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 98:S174

We developed a rapid and simple Q-tip-based oxalic acid method to evaluate hosta cultivars for resistance to petiole rot caused by *S. rolfsii* var. *delphinii*. Petioles (with attached leaves) of 12- and 17-week-old greenhouse-grown hosta cultivars “Lemon Lime”, “Munchkin”, “Tardiflora”, “Pearl Lake”, “KiKuti”, “Zounds”, “Honeybell”, “Gold Drop”, and “Halcyon” were severed at the crown with a sterile scissors. After a petiole was placed on hardware cloth, the end of a Q-tip was placed at the juncture with the leaf, and 20 µl of oxalic acid (50 mM) was dispensed onto the cotton of the Q-tip. Non-treated control petioles received 20 µl sterilized distilled water on Q-tips. Q-tips were secured to leaves and hardware cloth with rubber bands. Immediately after treatment, petioles and hardware cloth were placed in a dew chamber at 100% relative humidity and 27°C. Cultivars Lemon Lime, Munchkin, and Tardiflora rapidly developed bleached areas on petioles and leaves (highly susceptible), whereas Halcyon and Gold Drop developed symptoms much more slowly (highly resistant). The results were generally consistent with results of whole-plant inoculations in field and greenhouse tests, but the oxalic acid method required less than a week compared to 3 to 5 months for the whole-plant assays. The Q-tip-based oxalic acid method has potential to dramatically speed up identification of highly resistant cultivars for use in breeding programs.

DNA sequence evidence of the need for revision of taxonomic placement of plant-pathogenic *Sclerotium* species

Z. XU (1), M. L. Gleason (1), J. C. Batzer (1), T. C. Harrington (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 98:S174

Genetic evidence was used to assess the taxonomic placement of nine plant-pathogenic *Sclerotium* species. Partial sequences of rDNA large subunit (LSU) and internal transcribed spacer (ITS) regions were generated for isolates of *S. cepivorum*, *S. coffeicola*, *S. denigrans*, *S. hydrophilum*, *S. oryzae-sativae*, *S. perniciosum*, *S. rhizodes*, *S. rolfsii*, and *S. rolfsii* var. *delphinii*. Results of parsimony analysis indicated that *S. cepivorum*, *S. denigrans* and *S. perniciosum* should be reclassified as Ascomycota. The findings correct anecdotal information that indicated that *S. denigrans* and *S. perniciosum* belong to the Basidiomycota. Our study provided new insights into phylogenetic relationships of *Sclerotium* species as a whole and phylogenetic placement of species. Clarifying taxonomic status among *Sclerotium* species could pave the way for more effective management of the diseases they incite.

Construction of bioluminescent *Clavibacter michiganensis* subsp. *michiganensis*

X. XU (1), G. Rajashekara (2), S. A. Miller (1)
(1) Department of Plant Pathology, The Ohio State University, OARDC, 1680 Madison Ave., Wooster, OH, USA; (2) Food Animal Health Research Program, OARDC, The Ohio State University, Wooster, OH, USA
Phytopathology 98:S174

Clavibacter michiganensis subsp. *michiganensis* (Cmm), the pathogen of tomato bacterial canker, has caused severe economic losses in commercial tomato production worldwide. An important means of disease spread is through infected seeds and seedlings. In order to study the biology of seed to seedling transmission of Cmm, we constructed bioluminescent Cmm strains by transformation of the reporter gene, *lux*-operon, into Cmm. A promoterless *lux*-operon, originally derived from *Photorhabdus luminescence*, was linked downstream of a chloramphenicol exporter in a Cmm transposon mutagenesis vector, pKGT452Cbeta. The constructed vector pXX2 carrying the cassette *Cmxr::luxABCDE::Tn1409* was designed to integrate randomly into the Cmm chromosome allowing real-time imaging of the dynamics of seed to seedling transmission of Cmm. After repeated electroporation attempts with pXX2, several bioluminescent Cmm strains were obtained. A virulent, stable, constitutively bioluminescent strain will be used to study epiphytic and pathogenic colonization of tomato seeds and seedlings by Cmm.

Host-derived RNA interference analyses of selected parasitism genes of the root-knot nematode *Meloidogyne incognita*

B. XUE (2), G. Huang (3), T. J. Baum (1), R. S. Hussey (3), E. L. Davis (2)
(1) Iowa State University, Ames, IA, USA; (2) NC State University, Raleigh, NC, USA; (3) University of Georgia, Athens, GA, USA
Phytopathology 98:S174

Greater than fifty putative parasitism genes have been isolated from the esophageal gland cells of the root-knot nematode *Meloidogyne incognita* that

encode proteins potentially secreted into plants through the nematode stylet to promote host root infection. Functional analyses of ten of the *M. incognita* parasitism genes that encode novel proteins designated 2G10, 4D03, 8D05, 10G02, 16E05, 34F06, 9H10, 19F07, 35A02, and 30HO7 are under investigation using plant host-derived RNA interference (RNAi) gene silencing assays in transgenic *Arabidopsis thaliana*. Refinement of an Arabidopsis root-knot nematode infection assay in a sand+soil mixture has provided a convenient and reliable system that could facilitate the functional analysis of parasitism genes by RNAi. An inoculation rate of 1000 eggs yields approximately 10 and 30 galls per wild-type Arabidopsis root system in the sand+soil assay in 4 and 8 weeks after inoculation respectively, providing a substantial baseline of infection for comparisons of RNAi treatment affects. At least one Arabidopsis line that expressed RNAi to parasitism gene 4D03, 8D05,10G02, or 35A02 significantly reduced nematode-induced root galling compared to controls, suggesting the potential to develop root-knot nematode resistance through parasitism gene silencing.

Screen of antagonistic bacteria against *Ralstonia solanacearum*

Q. XUE (1), J. Guo (1)
(1) Plant Protection, Nanjing, Jiangsu, China
Phytopathology 98:S175

Bacterial wilt caused by *Ralstonia solanacearum* is a serious threat for agricultural production in many provinces in China. In this study bacterial strains isolated from different environment were screened as potential biocontrol agents according to antagonistic tests on plates and experiments in greenhouse and field. Beforetime several bacterial isolates, such as ZAg, ZBts, and AR156 and so on, were chosen because of their *in vitro* antagonistic activity to only one *R. solanacearum* strain. However, based on the diversity analysis of *R. solanacearum* isolates from China, we found that even the same antagonist can show different antagonistic activity to diverse *R. solanacearum* strains. Strain Zs36 identified as Serratia spp. was selected because of its different inhibition to various 29 *R. solanacearum* strains. When 8 pathogen strains were chosen to do greenhouse experiments, the results indicated that the biocontrol efficacy of Zs36 to these *R. solanacearum* strains were between 19% and 70%. When it was applied together with other bacteria strains, the biocontrol efficacy against bacterial wilt of the mixture can reach 100%. This mixture controlled Phytophthora blight and root-knot nematode disease in a good efficiency as well. Furthermore, they can promote growth of tomato, pepper and cucumber and other plants. Besides, Zs36 colonized rhizosphere of plant perfectly. On the 14th day after seed inoculation, strain Zs36 colonized with the concentration of 5.05 × 107 cfu per gram rhizosphere soil when the concentration of the inoculum was around 2 × 109 cfu/ml. DGGE analysis for bacterial community of rhizosphere of plant treated with Zs36 showed that it had lesser effect on indigenous bacteria community. These work showed that Zs36 has a good commercial potential.

Characterization of *Geotrichum candidum* causing sour rot of peaches and nectarines in California

M. A. YAGHMOUR (1), P. Inderbitzin (1), R. M. Bostock (1), T. J. Michailides (1)
(1) University of California-Davis
Phytopathology 98:S175

Geotrichum candidum has emerged recently as the principal causal agent of sour rot in peaches and nectarines in California, responsible for major losses to some growers. Phylogenetic analysis of two genetic loci was used to assess the genetic diversity of *G. candidum* among isolates obtained from soil, leaves, fruit, and packing houses. Sequences corresponding to the internal transcribed regions 1 and 2 and the gene encoding the 5.8S ribosomal RNA subunit were amplified by PCR using universal primers. Specific primers also were designed with introns in the beta-tubulin gene 1 based on alignments with *Geotrichum citri-aurantii*, *Saccharomyces cerevisiae*, and *Pichia stipitis*. Sequences of California isolates of *G. candidum* were highly variable within the nuclear ribosomal cluster (ITS) but, unlike other fungi, there was little variation among beta-tubulin sequences from the isolates. Two type collection isolates, *G. candidum* mating types A1 and A2, were included in this study and shown to be distinct from isolates causing sour rot on peaches and nectarines. Pathogenicity tests confirmed virulence on nectarine fruit for all isolates collected from California.

The efficacy of methyl bromide and alternatives on *Agrobacterium tumefaciens* and *Phytophthora cactorum*

L. E. YAKABE (1), S. R. Parker (1), D. A. Kluepfel (1)
(1) USDA, ARS, Crops Pathology Genetics Research Unit, Davis, CA, USA
Phytopathology 98:S175

The presence of *Phytophthora* species, causal agents of root and crown rot, and *Agrobacterium tumefaciens*, causal agent of crown gall, diminishes the value of nursery walnut stock and reduces the yield and lifespan of production orchards. Current management strategies for these diseases rely on pre-plant fumigation with methyl bromide and 1,3-dichloropropene. While *Phytophthora* root and crown rots are effectively controlled, crown gall control has been inconsistent. In *in vitro* assays, methyl bromide, chloropicrin, iodomethane, metam sodium and dazomet eliminated *A. tumefaciens* and, *Phytophthora cactorum* populations in soil while 1,3-dichloropropene only reduced populations by 89% and 96%, respectively. Five days after treatment, methyl bromide had significantly reduced general aerobic bacterial populations while its alternatives did not suppress microbial populations below levels found in untreated soils. Data will be presented on the ability of *A. tumefaciens* to recolonize: 1) methyl bromide treated, 2) Telone C35 treated, 3) thrice autoclaved, and 4) untreated field soils. These results may be useful in understanding the inconsistent nature of crown gall control often observed in field conditions.

Effects of *ced-9* antisense expressing in transgenic tobacco plants on *Meloidogyne incognita*

F. YAMAMOTO (1), T. Padukkavidana (2), G. W. Polack (1), A. Calderon-Urrea (1)
(1) California State University, Fresno, Department of Biology, 2555 E. San Ramon Avenue M/S SB73, Fresno, CA 93740-8034; (2) Graduate School, Yale University, New Haven, CT, USA
Phytopathology 98:S175

As an alternative to using pesticides to control nematodes, we are exploring the possibility of using transgenic plants expressing nematode programmed cell death genes to control nematode infestation. We hypothesize that knocking down *ced-9*-like genes of plant parasitic nematodes using antisense RNA, will limit their proliferation and/or reproduction. Provided that there are similar *ced-9* sequences in parasitic nematodes, we predict that plants containing a reverse (antisense) *ced-9* gene would stimulate the programmed cell death pathway of parasitic nematodes, resulting in plant protection. We generated homozygous transgenic tobacco plants expressing either *ced-9-F* (*ced-9* gene clones in the sense orientation) or *ced-9-R* (*ced-9* gene cloned in the antisense orientation). Selected *ced-9-R* and *ced-9-F* transgenic tobacco lines, both expressing high levels of the transgene (as determined by competitive RT-PCR) and no other phenotypic effect, were tested for resistance to *Meloidogyne incognita* (Root-Knot Nematode-RKN) by measuring gall formation, size of galls generated, and J2 hatching ability. The means of number of gall formation did not exhibit any statistical difference between transgenic and wild-type tobacco plants. Gall size was smaller, however, in transgenic *ced-9-R* or *ced-9-F* than in control plants. Furthermore, hatching ratios were low in *ced-9-R* transgenic plant lines, by approximately 50%, when compared to *ced-9-F* or control plants. Results from these experiments suggest that expression of either *ced-9-R* or *ced-9-F* genes in tobacco plants induced prevention of *M. incognita* proliferation. However, *ced-9-F* expressing plants prevent the proliferation by limiting the size of galls formed, while *ced-9-R* expressing plants do so by both limiting the size of galls formed and by preventing embryo hatching. We speculate that the hatching prevention in the *ced-9-R* expressing plants is due to the action on a *ced-9* like sequence during embryogenesis of *M. incognita* taking place in the transgenic plant.

Detection and discrimination of *Pratylenchus neglectus* and *P. thornei* in DNA extracts from soil

G. YAN (1), R. W. Smiley (1), P. A. Okubara (2)
(1) Oregon State University, Columbia Basin Agricultural Research Center, P.O. Box 370, Pendleton, OR 97801, USA; (2) USDA, Root Disease and Biological Control Research Unit, Pullman, WA, USA
Phytopathology 98:S175

The root-lesion nematodes *Pratylenchus neglectus* and *P. thornei* are the most damaging plant-parasitic nematodes in Pacific Northwest dryland wheat fields. Traditional methods of identifying these two species rely on the use of a nematode-extracting technique and microscopic observation. However, microscopic identification is time-consuming and frequently unreliable due to difficulties in distinguishing the key morphological characteristics. A species-specific polymerase chain reaction (PCR) method was developed to detect and identify *P. neglectus* and *P. thornei* in soil. A primer set was designed from *Pratylenchus* 26S rRNA gene sequences of the D3 expansion domain and their specificity was confirmed with plant-parasitic and non-parasitic nematodes typically present in the soil communities, and with six fungal species commonly associated with wheat root rot. DNA obtained using a commercially available kit and an inexpensive method developed in our laboratory gave comparable amplification. Optimized PCR conditions were established and the two species were differentiated by PCR products of 144 bp for *P. neglectus* and 288 bp for *P. thornei*. With this assay we were able to detect a single juvenile in 1 gram of sterilized, inoculated soil. Examination of 30 field soil samples revealed that this method was applicable to a range of soils naturally infested with these two pathogens in Oregon. This new PCR-based method is rapid, reliable, and inexpensive, eliminates the use of

nematode-extraction and morphological identification techniques, and can be used as a rapid diagnostic tool in commercial and research applications for disease forecast and management. Protocols developed in this study are being adapted for use in real-time PCR applications, which would also enable us to quantify *P. neglectus* and *P. thornei* from soil.

First detection of the cereal cyst nematode *Heterodera filipjevi* in North America

G. YAN (1), R. M. Smiley (1)
(1) Oregon State University, Columbia Basin Agricultural Research Center, P.O. Box 370, Pendleton, OR 97801, USA
Phytopathology 98:S176

Heterodera avenae, *H. filipjevi* and *H. latipons* are recognized as the most economically important cyst nematodes affecting cereals worldwide. Until recently, cyst nematodes from wheat and barley fields in the Pacific Northwest (PNW) were identified as homogenous populations of *H. avenae*. During PCR-RFLP identification of *H. avenae* collected in the PNW, cysts from a winter wheat field near Imbler (Union County), Oregon revealed a restriction pattern matching that of *H. filipjevi* rather than *H. avenae* when digested with four endonucleases *Taq*I, *Hinf*I, *Pst*I, and *Hae*III The pattern was also different than that of *H. latipons* when digested with *Taq*I and *Hae*III, ruling out the possibility that the cysts from Imbler could be *H. latipons*. Comparisons of cysts and vulval cones under a microscope revealed a characteristic morphological difference between the Imbler cysts and known specimens of *H. avenae*. A distinct underbridge with bifurcated arms was present in vulval cones of the Imbler cysts whereas no underbridge was found in those of *H. avenae*. Fenestral pattern of the Imbler cysts was more horseshoe-shaped than symmetrically round; cyst cuticle was lighter in color; eggs were more easily observed through the cyst cuticle; and eggs hatched much more readily. These characteristics each conform to distinctions between *H. filipjevi* and *H. avenae*, supporting the hypothesis that the cysts from Imbler are *H. filipjevi*, a species not previously reported in the PNW. Our observations were confirmed by USDA nematode taxonomists through both morphometric and molecular analyses. Detection of *H. filipjevi* in Oregon represents a new record for the presence of this species in North America.

Evaluation of rhizospheric fluorescent *Pseudomonas* for the growth promotion of alfalfa plants

M. Yanes (3), L. De La Fuente (1), N. A. ALTIER (2), A. Arias (3)
(1) Department of Plant Pathology, Cornell University, Geneva, NY, USA; (2) Instituto Nacional de Investigación Agropecuaria, INIA Las Brujas, Canelones, Uruguay; (3) Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
Phytopathology 98:S176

Alfalfa (*Medicago sativa* L.) is used as quality feed for livestock in the Uruguayan agricultural system. Crop establishment is often affected by seedlings diseases incited by soil-borne fungal pathogens, which cause important commercial losses. Biological control mediated by fluorescent *Pseudomonas* offers an effective and environmentally friendly strategy for the suppression of these diseases. A collection of 701 rhizospheric fluorescent *Pseudomonas* isolated from healthy alfalfa plants was obtained from three locations in Uruguay. The isolates were evaluated to determine the direct and indirect plant growth promotion mechanisms commonly found in *Pseudomonas* spp. *In vitro* antagonism against *Pythium debaryanum*, the presence of antibiotic biosynthetic genes, and the production of biosurfactant compounds, cellulases and proteases, were evaluated. Also, the ability to solubilize inorganic phosphate was assessed. The proportion of isolates showing these characteristics varied according to the soil background of the location they came from. Genotypic characterization by rep-PCR of those isolates collected from soils bearing alfalfa crop history showed related genotypes. When cluster analysis was applied, the genotypes of isolates with the same phenotypic characteristics previously evaluated clustered together. The ability of 101 selected isolates to suppress alfalfa damping-off caused by *P. debaryanum* was evaluated on growth chamber assays. Five isolates showed a significant control effect of damping-off while four of them also exhibited a promotion effect on alfalfa biomass. These pseudomonads isolates are good candidates for biocontrol agents based inoculants.

Induction of necessary host factors, the ribosomal proteins, by plant viruses

C. YANG (1), S. Whitham (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 98:S176

Potyvirus infection results in increased mRNA transcripts of host ribosomal protein (r-protein). For example, the mRNAs of at least 69 Arabidopsis thaliana r-protein genes are induced concomitantly with Turnip mosaic virus (TuMV) accumulation. This observation led us to investigate whether r-proteins are host factors for plant virus infection. Because many, if not all, r-proteins are essential to the host, virus-induced gene silencing (VIGS) was used to transiently silence the expression of RPS6, RPL19, RPL13, RPL7, and RPS2 in Nicotiana benthamiana plants. Silencing these r-protein genes resulted in arrested plant growth, altered shape of the newest leaves, and chlorosis. Plants with these phenotypes remained viable and were inoculated with TuMV and Tobacco mosaic virus (TMV) tagged with GFP. The accumulation of TuMV and TMV was strongly dependent on RPL19, RPL13, RPL7, and RPS2. Interestingly, TMV was able to accumulate efficiently in RPS6-silenced plants in which TuMV. To further investigate the specificity of these observations, RPS6- and RPL19-silenced plants were also inoculated with Tomato bushy stunt virus (TBSV), and the viral infection was nearly abolished in both types of plants. These results demonstrate that TuMV and TBSV have a strong requirement for RPS6 and other r-proteins, but TMV does not require RPS6 for its accumulation. These results are particularly interesting when considered in the context of the translation strategies of these three viruses – TuMV and TBSV are cap-independent, whereas TMV RNAs have a 7-methly guanosine cap at their 5′ ends.

Ethylene pathway and disease resistance in rice are negatively regulated by a stress-responsive MAP kinase

Y. Yang (1), T. A. Bailey (2), J. Chen (1), E. E. HELLIWELL (1), X. Zhou (2)
(1) Department of Plant Pathology, Pennsylvania State University, University Park, PA 16802, USA; (2) Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA
Phytopathology 98:S176

A stress-inducible rice MAP kinase (OsMPK5) was previously shown to negatively modulate disease resistance and defense gene expression; however, its underlying signaling mechanism is unknown. In light of increasing evidence that ethylene (ET) and its antagonistic interaction with abscisic acid (ABA) may play important roles in rice defense response against the blast fungus (*Magnaporthe oryzae*), we have examined the potential role of OsMPK5 in regulating ethylene biosynthesis and signal transduction using a combination of molecular, biochemical and transgenic approaches. Transgenic analysis showed that suppression of OsMPK5 by RNA interference decreased the endogenous ABA level, but significantly increased ethylene level and rice blast resistance. Interestingly, a pathogen-inducible ET-biosynthetic enzyme (1-aminocyclopropane-1-carboxylic acid synthase 2, OsACS2) was found to contain conserved phosphorylation sites in its C-terminus, which may be phosphorylated by OsMPK5. In addition, *in vitro* binding and phosphorylation assays demonstrated that OsMPK5 physically interacted with and phosphorylated rice EIL1 transcription factor, a key signaling component of ET signal transduction. These data suggest that OsMPK5 may negatively modulate both ET biosynthesis and signal transduction that in turn regulates downstream rice defense gene expression and disease resistance.

The importance of geographical location of field trials in evaluating new fungicides against *Mycosphaerella graminicola*

C. YAO (1), D. Young (1), C. Klittich (1), A. Meitl (1)
(1) Dow AgroSciences, Indianapolis, IN, USA
Phytopathology 98:S176

Leaf blotch of wheat caused by *Mycosphaerella graminicola* is an important constraint to intensive wheat production. Resistance development to strobilurins together with shift in sensitivity to triazoles in Europe have seriously limited options for control of this disease. We compared *in-vitro* and *in-planta* sensitivity of a set of European isolates with one New Zealand (NZL-12), and one U.S. isolate (USA-184) to strobilurins, epoxiconazole, and an experimental compound with a novel mode of action. The European set included both strobilurin-sensitive and resistant isolates. However, all European isolates were significantly more tolerant to epoxiconazole in greenhouse and *in vitro* assays than NZL-12 and USA-184. In their sensitivity to epoxiconazole and strobilurins, the New Zealand and U.S. strains may be similar to European strains from the pre- triazole/strobilurin era. In contrast to strobilurins and epoxiconazole, the experimental chemistry showed comparable activity against the isolates from all geographies. In field tests, the experimental chemistry provided a similar level of control in both New Zealand and Europe. In New Zealand where strobilurins and azoles have not been used intensively, both azoxystrobin and epoxiconazole demonstrated excellent control and were better than the experimental compound. However, in European trials the experimental compound provided comparable control to epoxiconazole, and azoxystrobin was practically inactive against the resistant population. These data highlight the importance of geographical location of field trials in evaluating new fungicides. Although Europe is the most important market for cereal fungicides, field trials should also be conducted in regions lacking resistance to commercial fungicides to benchmark new chemistry against the best historic standards.

The bacterial phytotoxin coronatine targets the Arabidopsis SCF^{COI1}–JAZ protein complex

J. YAO (1), C. Mecey (1), M. Melotto (1), W. Zeng (1), S. He (1)
(1) Department of Energy-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA
Phytopathology 98:S177

The bacterial phytotoxin coronatine is produced by several pathovars of the phytopathogenic bacterium *Pseudomonas syringae* and plays important roles in pathogen – host interactions. However, the host targets of this toxin have not been identified. Coronatine structurally mimics the plant hormone jasmonic acid (JA). Recently, an important step in JA signaling pathway has been discovered: JA induces SCF^{COI1} (an E3 ubiquitin ligase)-dependent degradation of JAZ proteins, which are repressors of JA signaling. Here, we show that coronatine promotes the physical interaction between the COI1 (a subunit of SCF^{COI1}) and JAZ proteins. In yeast two hybrid assays, the interaction between COI1 and multiple JAZ proteins depends on coronatine, but not its two biosynthetic precursor coronafacic acid or coronamic acid. We also successfully pulled down the COI1 protein from Arabidopsis leaf extracts in a coronatine-dependent manner by using *E. coli*-expressed JAZ protein as bait. Collectively, these results suggest that coronatine facilitates the recruitment of JAZ repressor proteins by the SCF^{COI1} E3 ubiquitin ligase.

Assessment of bacteria from apple leaves by culture-dependent and culture-independent methods

E. YASHIRO (1), R. Spear (1), K. Clinton-Cirocco (1), P. McManus (1)
(1) University of Wisconsin - Madison, Madison, WI, USA
Phytopathology 98:S177

It is well established that about 1% of bacteria in soil are recovered by culturing on standard media. We have discovered a similar phenomenon on apple leaves, which implies that a larger and more diverse array of bacteria might harbor bactericide-resistance genes than has been documented by culturing. We identified bacteria washed from apple leaves by isolation on tryptic soy agar and by analysis of 16S rDNA libraries built from total bacteria. Bacteria cultured from a site where copper and streptomycin have been used consisted of members of the Actinomycetales (48%), Pseudomonadales (14%) and Sphingomonadales (38%), whereas isolates from a site where bactericides have not been used consisted of Actinomycetales (21%), Rhizobiales (32%), and Sphingomonadales (47%). When TSA was amended with streptomycin, isolates from the treated site maintained a similar bacterial richness and abundance, but only members of Sphingomonadales could be isolated from the non-treated leaves. DNA cloned from leaf wash represented at least six distinct taxa within the Bacteroidetes and Proteobacteria. Many clones contained sequences related to known plant-associated or airborne bacteria; however, none of those within the Bacteroidetes matched sequences in GenBank by more than 97%. Also more than 70% of the cloned sequences from the two sites grouped within Sphingomonadales. We conclude that culture-independent methods reveal a greater number of bacteria and a different array of taxa than does culturing.

Growth rate and temperature tolerance of diverse *Trichoderma koningiopsis* isolates

I. E. YATES (2), S. Chambliss-Bush (2), G. Samuels (1), D. Sparks (3)
(1) USDA, ARS, Systematic Botany and Mycology Laboratory; (2) USDA, ARS, TMRU; (3) University of Georgia, Department of Horticulture
Phytopathology 98:S177

The fungus, *Fusarium verticillioides*, colonizes corn plants causing problems in crop production from plant disease and in human and animal health from mycotoxin contamination of food and feed products. The negative aspects of this fungal/plant interaction is usually more common in warmer climates in the southeastern United States than in more northerly regions. Methodology has not been developed to prevent the deleterious effects of this fungal/plant interaction. A *Trichoderma* sp. was isolated from corn plants grown in the southeastern United States and has been demonstrated to inhibit *F. verticillioides* growth and mycotoxin production on corn kernels. This isolate has been identified as *T. koningiopsis*, a common predominantly tropical species. The purpose of the current study was to compare growth rate and temperature tolerance of the *T. koningiopsis* from Georgia to isolates from diverse geographic sites around the globe. Populations examined originated from Brazil, Canada, Ecuador, Germany, and Kentucky in the United States. Growth rate and temperature tolerance were examined by daily measurements for seven days at temperatures increasing from 15°C to 35°C in 5°C increments. Both the growth rate and temperature tolerance appeared more expansive for isolates other than the one from Georgia. Thus, *T. koningiopsis* isolates originating from other geographic locations merit evaluation for their biocontrol efficacy in controlling *F. verticillioides* growth and mycotoxin production on corn grown in the southeastern United States.

Biological control of bacterial spot and anthracnose of pepper by using *Bacillus megaterium* 22-5

C. YEON (1), H. Kim (1)
(1) Dept. of Plant Medicine, Chungbuk National University, 12 Gaeshin-dong, Cheongju, Chungbuk 361-763, Korea
Phytopathology 98:S177

The bacterial spot and anthracnose are economically important diseases on pepper and the diseases are difficult to control. For selecting effective biological control agents for the bacterial spot and the anthracnose of pepper, total 444 bacteria were isolated from the rhizosphere of pepper. The 14 bacterial isolates from 444 isolates showed a good activity of bacterial spot in greenhouse assay. By further screening for suppression on anthracnose in laboratory, greenhouse and field, the isolate 22-5 was selected. Nucleotide sequence of 16S rDNA of the bacterium indicated that it is *Bacillus megaterium*. Control value of the bacterial suspension on bacterial spot disease was 66.7% by soil drench treatment in a greenhouse test. The 63.6–95.5% of anthracnose was decreased on the treated detached pepper by the bacterial suspension depending on treatment time. The 4 times treatments including soil drench and spray of bacterial suspension controlled 50.5 – 94.1% of anthracnose in field. These results suggest that a very effective biological control formulation on the bacterial spot and anthracnose on pepper could be developed with B. megaterium 22-5.

Potential of phosphorous acid-containing products for control of Phytophthora blight on squash

J. YIN (1), K. L. Jackson (1), A. S. Csinos (1), P. Ji (1)
(1) University of Georgia, Tifton, GA, USA
Phytopathology 98:S177

The potential of several phosphorous acid-containing products for control of Phytophthora blight, incited by *Phytophthora capsici*, was evaluated by comparing their efficacy in suppressing the pathogen in *in vitro* studies and disease development in greenhouse assays. The phosphorous acid-containing products, including ProPhyt, K-Phite, Lexx-A-Phos, Agri-Fos and NutriPhite, were applied at the same concentrations based on phosphorous acid equivalent. EC_{50} values of the phosphorous acid-containing products in inhibiting mycelial growth on PDA ranged from 80 to 370 ppm based on phosphorous acid equivalent, and from 80 to 900 ppm in suppressing sporangia production. None of the phosphorous acid-containing products at the concentrations used was effective in reducing germination rates of zoospores, while zoospore germ-tube elongation was affected by higher concentrations of the products. EC_{50} values of mefenoxam (Ridomil Gold) in suppression of mycelial growth and sporangia production were lower compared to the phosphorous acid-containing products, and zoospore germination was inhibited completely by mefenoxam. In greenhouse studies, the products were used to treat soil artificially infested with *P. capsici* and squash seedlings were transplanted in the treated soil. Mefenoxam and some phosphorous acid-containing products reduced disease on squash compared with the non-treated control with mefenoxam being the most effective. Soil and root drench using the phosphorous acid-containing products or mefenoxam did not provide systemic protection of squash seedlings against *P. capsici* that was inoculated onto the leaves, suggesting that a direct effect of these products on the pathogen probably contributed more than induced host resistance in disease control. These studies indicated that some phosphorous acid-containing products had the potential to suppress *P. capsici* but were less effective than mefenoxam. Development of integrated approaches, rather than use of these products alone, would be beneficial for more efficient management of the disease.

Generation and analysis of expression sequence tags from haustoria of the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici*

C. YIN (3), X. Chen (2), Z. Kang (1), S. Hulbert (3)
(1) Northwest A&F University, Yangling, Shaanxi, China; (2) USDA-ARS and Washington State University, Pullman, WA, USA; (3) Washington State University, Pullman, WA, USA
Phytopathology 98:S177

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a destructive disease of wheat (*Triticum aestivum* L.) worldwide. Like other rust fungi, *Pst* forms haustoria, which are specialized structures that develop within host cells and are believed to play an essential role in nutrient uptake, vitamin synthesis, and the suppression of host defenses. Stripe rust haustoria were isolated from infected wheat leaves by ConA affinity chromatography and a haustorial cDNA library of the fungal pathogen was constructed. A total of 3,711 EST sequences with high quality were generated. Assembly of the sequences resulted in 1,253 unigenes, of which 1004 originated from singletons while 249 were represented by more than one EST. BlastX searches identified 86 (7%) of the unigenes were similar to proteins of known function (e-value < 1.00E-5), 190 (15%) were similar to proteins of unknown function, and 977 (78%) showed no significant matches. Seventy-nine of the unigenes were

predicted to code for proteins that were secreted from haustoria. Quantitative RT-PCR studies revealed that some of the cDNAs were specifically expressed in plants. Methods for transient expression of these secreted protein-like genes in wheat plants are under development.

Isolation and comparison of new *Lysobacter enzymogenes* strains for biological control traits

H. YIN (2), E. Caswell-Chen (1), G. Y. Yuen (2)
(1) University of California - Davis, Davis, CA, USA; (2) University of Nebraska - Lincoln, Lincoln, NE, USA
Phytopathology 98:S178

Biocontrol strains of *Lysobacter enzymogenes*, a bacterial species reported in soil and other habitats, exhibit varying levels of enzyme and antibiotic activities against plant pathogens. To begin assessing the distribution of *L. enzymogenes* and the diversity of strains within the diverse soil ecosystems in Nebraska, 37 soil samples were collected throughout Nebraska and putative *L. enzymogenes* strains were isolated through enrichment culturing with chitin as the carbon source. After 16S rDNA analysis of the putative strains, ten were identified to be *L. enymogenes*, all of them originating from one soil sample. Comparison of the new strains' biocontrol-related activities *in vitro* with those of known biocontrol strains and the species type strain revealed little variation among the ten new isolates and biocontrol strains in enzyme activity and antagonism against bacteria, fungi, and nematodes. The species type strain exhibited lower activities than the others despite similar growth. The results thus far suggest that *L. enzymogenes* is not widespread and there is little diversity among strains. We are attempting to confirm these findings by further isolations from soil and evaluation of strains for in planta biocontrol activity in addition to *in vitro* traits. Furthermore, we intend to confirm the presence or absence of *L. enzymogenes* in soils using PCR-based techniques. To this end, we have identified a primer specific to the genus *Lysobacter* by surveying all reported 16S rDNA sequences for *Lysobacter* spp.

Genetic diversity of *Citrus tristeza virus* isolates spreading in Central California

R. K. YOKOMI (2), M. Polek (1), M. Saponari (2)
(1) CDFA/CCTEA, Tulare, CA, USA; (2) USDA, ARS, SJVASC, Parlier, CA, USA
Phytopathology 98:S178

A rapid increase in trees infected with *Citrus tristeza virus* (CTV) was observed in several locations in Tulare, County, CA in 2007. Leaf and bark tissue were sampled from infected trees and used for molecular characterizations. Real-time RT-PCR using a universal CTV TaqMan probe detected all isolates. No reactions were obtained with a CTV-stem pitting TaqMan probe but several samples reacted to a TaqMan probe for California MCA13 positive isolates which do not induce decline. All isolates had a T30 genotype and SSCP analysis of the CP showed most isolates had a profile identical to the mild P81 isolate. However, three different SSCP patterns were observed. Sequencing of the CP gene showed at least two different isolates were present along with the common P81-like isolate. Pairwise alignments of these isolates shared only 91.5% and 90.7% nucleotide identity with mild isolates which suggests that they are genetically distinct isolates. Phylogenetic relationships indicated that one isolate was closely related to but distinct from the Florida T36 strain (94% nucleotide identity); whereas the other isolate was in a separate clade with a grapefruit stem-pitting isolate from Argentina (C269-6) and a sub-isolate (P108-35) of the Dekopon isolate, a virulent CTV strain intercepted in central California. Host range biocharacterization of these isolates are ongoing. We continue to monitor the spread and diversity of these CTV isolates in an effort to explain a trend of rapid spread even in some areas of the Central Valley where CTV-infected trees are consistently removed.

Transgenic rice plants expressing an active tobacco mitogen-activated protein kinase kinase induce multiple defense responses

S. Yoo (1), J. Jeong (1), D. Yang (1), Y. Kim (1), B. Cho (1), K. YANG (1)
(1) Department of Plant Biotechnology, Chonnam National University, Gwangju 500-757, Korea
Phytopathology 98:S178

Extensive studies have been performed about the functional understandings of almost MAPK genes in the dicotyledonous plants. NtMEK2, a tobacco MAPK kinase, is known as the upstream kinase of both salicylic acid-induced protein kinase (SIPK) and wounding-induced protein kinase (WIPK). Expression of $NtMEK2^{DD}$, a constitutively active mutant of NtMEK2, induced multiple defense responses in tobacco. However, little is known about the MAPK genes in rice, economically valuable monocotyledon plant. To understand the roles of rice MAPK cascade involved in disease resistance, transgenic rice plants were generated with an active or inactive mutant of NtMEK2 under the control of a steroid inducible promoter. The expression of $NtMEK2^{DD}$ in transgenic rice plants resulted in leads to HR-like cell death, which is preceded by the activation of endogenous 48-kDa MBP kinase. That MAPK is also activated by *Xanthomonas oryzae* pv. *oryzae*, the bacterial blight pathogen of rice. In addition, the prolonged activation of the MAPK induces the generation of hydrogen peroxide and control the expression of some of downstream MAPKs and defense-related genes of rice including pathogenesis-related genes, peroxidase and glutathione S-transferase. These results demonstrate that NtMEK2 is functionally interchangeable with rice MAPK kinase in activating downstream MAPK pathway involved in multiple defense responses in rice.

Thiophanate methyl-resistant *Colletotrichum cereale* isolates exhibiting amino acid substitutions in the beta-tubulin 2 gene

J. R. YOUNG (1), M. T. Peterson (1), F. P. Wong (2), K. de la Cerda (2)
(1) Mississippi State University, Mississippi State, MS, USA; (2) University of California, Riverside, Riverside, CA, USA
Phytopathology 98:S178

Colletotrichum cereale, the causal organism of anthracnose, has become a difficult pathogen to manage on creeping bentgrass putting greens in the southern U.S. Thiophanate methyl (TM) is a broad spectrum, single-site mode-of-action fungicide that has a high propensity to form TM-resistance within certain turfgrass pathogens. The *C. cereale* isolates infecting creeping bentgrass in the southern U.S. had not been examined to determine their sensitivity to TM. The objectives of this study were to determine if the *C. cereale* isolates collected in the southern U.S. were resistant to TM using *in vitro* bioassays and to identify amino acid point substitutions that lead to TM resistance. *In vitro* bioassays were performed in final concentrations of 0, 0.039, 0.156, 0.625, 2.5, and 10 ppm TM. All the *C. cereale* isolates collected from creeping bentgrass were resistant to TM at the discriminatory concentration of 10 ppm TM. A representative sample of *C. cereale* isolates was used to examine possible substitutions in the beta-tubulin 2 gene. Amino acid sequences were obtained from PCR-product of six *C. cereale* isolates. Five of the resistant isolates had an amino acid substitution from glutamic acid to alanine at amino acid position 198. The resistant isolate, FL A2-1, had an amino acid substitution from phenylalanine to tyrosine at amino acid position 200. These substitutions differ from those found in a separate study performed in California on *C. cereale* isolates, which resulted in all TM-resistant isolates having an amino acid substitution from glutamic acid to lysine at position 198.

Organisms associated with internally discolored horseradish roots

J. YU (1), M. Babadoost (1)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL, USA
Phytopathology 98:S178

This study was conducted to determine bacteria and fungi associated with the internally discolored horseradish roots. Horseradish root samples were collected from Illinois, Wisconsin, California, and Minnesota, and 40 roots from each sample were assayed for presence of bacteria and fungi. The roots were surface-sterilized by dipping in 6% sodium hypochlorite for 1 min followed by dipping in 95% ethanol concentration for 3 min, and then washed in sterile-distilled water three times. Root sections were cultured on acidified potato dextrose agar and nutrient agar. Bacterial and fungal colonies growing out of the cultured root sections were identified based on the morphology of the organisms, chemical tests, and PCR-based detection protocols. We have routinely isolated and identified the following organisms from internally discolored roots: *Verticillium dahliae*, *V. longisporum*, *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Trichoderma*, *Pseudomonas*, *Erwinia*, and *Bacillus* species. It is already known that *V. dahliae*, *V. longisporum*, and *F. solani* cause internal root discoloration in horseradish. But, the roles of other organisms associated with internally discolored roots have not been determined yet. We intend to determine pathogenicity of the isolated organisms on horseradish and interactions among them.

Implications of fungicide application delays on Asian soybean rust control

M. L. ZACCARON (1), J. L. de Oliveira (1), G. W. Fengler (1), B. A. Pontim (1), B. Zuntini (1), M. Mendes (1), R. Kobayashi (1), L. A. Bacchi (1), W. L. Gavassoni (1)
(1) Universidade Federal da Grande Dourados – Dourados, Brazil
Phytopathology 98:S178

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, is the most destructive disease of soybean. Among control strategies, chemical control is the most widely used by soybean growers in Brazil. Timing of fungicide spray is crucial to avoid yield losses due to ASR. Six experiments were conducted in Mato Grosso do Sul, Brazil, during the 2007 (cv. BRS 245RR) and 2008 (cv. CD 219RR) cropping seasons to assess the impact of delaying fungicide sprays on the efficacy of disease control and its impact on soybean yield. Experimental design was a randomized block with treatments involving fungicide spray beginning at the detection of the disease in the plots, and delays in the first, second and third sprays. Fungicides used in the experiments were: pyraclostrobin + epoxiconazole, azoxystrobin + cyproconazole,

trifloxystrobin + tebuconazole and picoxystrobyn + cyproconazole. Disease severity, defoliation and yield losses were greater in treatments with fungicide spray delay compared to sprays on the day that rust was detected. When fungicide applications were delayed in 10 days soybean yield was reduced in 10.8%. Losses due to disease could be greater than detected considering that ASR was first found when soybean growth stage was R5.

Gammabacteria associated to leaf chlorotic strikes in maize crop during seed production in Mexico

L. X. ZELAYA-MOLINA (2), H. V. Silva-Rojas (1), L. Cordova-Tellez (1), E. Valadez-Moctezuma (2), P. D. Esker (3)
(1) Colegio de Postgraduados, Texoco, Edo. de Mexico, Mexico; (2) Universidad Autonoma Chapingo, Texoco, Edo. de Mexico, Mexico; (3) University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 98:S179

During summer of 2005–2007, in six different seed maize production plots in Mexico State, symptoms of leaf chlorotic streakes were observed. The initial symptoms were detected in seedlings of 15 days old after emerging, the disease was monitored during all the crop season until production, and the effect of the disease in the yield was obvious, since the plants did not produce ears of good quality. At the first stages of the crop, from symptomatic leaves, small pieces of 1 cm^2 were taken from the convergence of diseased and healthy tissues, and placed on CPG and Chalk media. Forty eight different isolates were obtained, and characterized by molecular techniques. The 16S rDNA was sequenced from all the strains, and the phylogenetic analysis showed that they belonged to four different genera of Gammabacteria: *Herbaspirillum*, *Pseudomonas*, *Stenotrophomonas*, and at least two species of *Pantoea*. Multilocus analysis using *gyr*B, *rec*A, Tuf, and *car*A genes, specified their identification as *Pantoea agglomerans* species complex, *P. ananatis*, *Pseudomonas r*hizosphaerae, *S. maltophilia*, and *Herbaspirilum putei*. Pathogenicity test was carried out with *P. ananatis* and *P. agglomerans* strains, inoculating seedlings of two weeks old, and symptoms were reproduced. These results indicate that several Gammabacteria are associated to leaf chlorotic strikes on maize, as well as the different ecological niches they inhabit, and let them live inside the same plant are until now unknown.

Head-to-head comparisons of sensitivity and specificity among 5 real-time PCR assays diagnostic for *Phytophthora ramorum*

K. A. ZELLER (1), R. M. DeVries (1), L. Levy (1)
(1) USDA-APHIS-PPQ-CPHST, National Plant Germplasm and Biotechnology Laboratory, Beltsville, MD 20705
Phytopathology 98:S179

In response to Stakeholder requests, we adapted and validated five alternative Real-time PCR diagnostic assays for *Phytophthora ramorum* developed by other laboratories that target DNA loci in the ribosomal repeats, mitochondrial DNA, and in individual single-copy genetic loci for use in the Cepheid Smartcycler™. We have compared relative sensitivity and specificity of the methods by testing on a common set of >130 previously diagnosed environmental samples. Three of the 5 tested diagnostic methods failed to cross-react with DNAs from closely related *Phytophthora foliorum*, *P. hibernalis* or *P. lateralis*. However, each of these three methods also displayed lower sensitivity for target DNA of *P. ramorum* than do the validated conventional nested PCR assay and ITS-targeting Real-time PCR assay. Two of the three methods (mitochondrial *Cox* locus, and genomic Elicitin locus) are able to reliably detect *P. ramorum* DNAs to >50fg of target DNA. When combined with the currently used ITS-targeting Real-time PCR assay, either of these two Real-time PCRs should reduce the frequency of false positive results on initial tests of environmental samples for *P. ramorum*. The current ITS and Elicitin Real-time assays are multiplexed with internal control amplicons for mitochondrial and genomic DNAs, respectively, that replicate the internal controls in the current diagnostic system that make this combination desirable.

Silicon: Virus friend or foe?

W. L. ZELLNER (1), S. M. Leisner (1)
(1) University of Toledo
Phytopathology 98:S179

Silicon is a beneficial element that aids plant resistance to certain fungal and bacterial pathogens, such as powdery mildew and bacteria spotted wilt, respectively. However, the effects of silicon on viral infections are poorly understood. To develop a model system for these studies, ten *Arabidopsis thaliana* ecotypes were inoculated with *Tobacco ringspot nepovirus* (TRSV) to determine infectivity. One, three, and six ecotypes were resistant, susceptible, and tolerant, respectively. *A. thaliana* Sf-1, a TRSV susceptible ecotype, was then grown hydroponically, inoculated with TRSV, and supplemented with soluble silicon at 0.1 mM or 1.0 mM. Surprisingly, 1.0 mM silicon treatment resulted in a higher percentage of symptomatic plants compared to those amended with 0.1 mM silicon. *Nicotiana tabacum* is also susceptible to TRSV infection and was used as a second host for our study. TRSV symptoms on hydroponically-grown *N. tabacum* leaves amended with 0.1 mM soluble silica covered more surface area than leaves of plants grown under higher silicon concentrations, similar to previous reports on silicon-aided resistance. Our results suggest that silicon amendments aid virus resistance in a host-specific manner.

Control of white mold in soybean with biocontrol agents

W. ZENG (1), W. Kirk (1), R. Hammerschmidt (1), J. Hao (1)
(1) Michigan State University
Phytopathology 98:S179

Biocontrol agents, *Coniothyrium minitans* (Contans), *Bacillus subtilis* (Serenade) and *Trichoderma harzianum* (Plantshield) were evaluated for the control of soybean white mold (*Sclerotinia sclerotiorum*) in controlled environment chambers, the greenhouse, and the field. Sclerotia of *S. Sclerotiorum* for all experiments were produced aseptically on potato cubes. In the chamber and greenhouse experiments, 25 sclerotia per 1-L pot were mixed in potting soil, which was then treated with the biocontrol agents in water solutions at various rates. Soybeans (Olympus) were planted 4 wk after soil treatments. After 8 wk, sclerotia were retrieved and determined for viability. *C. minitans* and *T. harzianum* significantly reduced the number and viability of sclerotia, decreased apothecial production, and enhanced plant growth. The efficacy of *C. minitans* and *T. harzianum* on survival of sclerotia increased with their dose rate increasing except for *C. minitans* at 1 g/L soil. The optimal dosage of *B. subtilis* that limited apothecial germination was 1 g/L soil. For the field experiment, 3 × 3 m^2 plots were established and sclerotia were spread 2 wk before planting at 50 sclerotia/m^2. *C. minitans*, *B. subtilis* and *T. harzianum* were applied the day after planting at 2.2 kg/ha 4.4 kg/ha 0.35 kg/ha, respectively. White mold severity was evaluated at harvest. *C. minitans* and *T. harzianum* significantly reduced the number of viable sclerotia. *B. subtilis* had no significant effect.

The distribution of mating type and sexual status in Chinese rice blast populations

J. ZENG (1), S. Feng (1), L. Wang (1), F. Lin (1), J. Cai (1), Q. Pan (1)
(1) College of Natural Resources & Environment, South China Agricultural University, Guangzhou 510642, China
Phytopathology 98:S179

A collection of 520 field isolates of rice blast (Magnaporthe grisea), originating from five provinces in China, was tested for their mating type and sexual and fertility status. One of the two tester sets comprised isolates collected from barley and the other from rice. Two mating types (MAT1-1 and MAT1-2) were identified among the 443 fertile isolates. The two mating types were roughly in balance with one another in the southwestern region, but a single (but different) one predominated in the southeastern and southern regions. Male only fertile isolates were the most common, and only a few hermaphroditic and no female only fertile isolates were detected. The fertility level of the isolates varied, with those from Jiangsu being the most fertile, and those from Fujian the least. The mating capacity of the testers collected from barley was overall higher than that of the testers collected from rice, but this was because the MAT1-2 testers differed very markedly from one another (the mating capacities of the two MAT1-1 testers were similar to one another). We have proposed a likely model for the evolution and dispersal of rice blast in China.

Field evaluation of hair waste on yields of fresh market tomatoes in south Florida

S. ZHANG (1), T. L. White (1)
(1) TREC, University of Florida, IFAS, Homestead, FL
Phytopathology 98:S179

A field trial was conducted to evaluate hair waste for its potential to increase yields of fresh market tomatoes in south Florida. Tomato seedlings (cv. 'Sanibel') were grown in the greenhouse to 4 inches tall before being transplanted into fields. Hair mats, the product fabricated and distributed by SmartGrow (Florida City, FL) using hair waste, were placed on the top of the soil around the stem base and under the plastic mulch 3 weeks after transplanting (WAT). The experiment included a treatment with the hair product and a nontreated control with 6 replicates for each treatment, 30 plants per replicate. Two harvests were completed at 12 WAT and 14 WAT, respectively. At the first harvest, the treatment with hair mats increased the marketable yield of tomato by an average of 12.5% compared to the nontreated control, although this difference was not significant. Data from the second harvest indicated that the yields of the treated and control plots were very similar. No significant difference was observed between the treatment and control in the incidence of *tomato yellow leaf curl virus* and plant growth measured by plant height at 7 WAT. However, treatment with hair mats tended to increase the number of fruit sets per plant by 28.8%, albeit not significantly (P = 0.0529). Results from this study indicated that hair waste

may be useful in increasing yields of fresh market tomatoes, and further investigations are warranted to confirm this preliminary finding and to improve the treatment.

Preliminary assessment of PGPR, acibenzolar and silicon for their effects on growth and diseases of tomatoes

S. ZHANG (1), T. L. White (1), W. Klassen (1)
(1) TREC, University of Florida, IFAS, Homestead, FL
Phytopathology 98:S180

Plant growth-promoting rhizobacteria (PGPR) have been studied extensively for their ability to improve plant growth and to elicit disease resistance against fungal, bacterial and viral pathogens in a variety of crops. Induced resistance by acibenzolar-S-methyl has also been reported in various plants for control of plant pathogens. It is believed that application of silicon increases resistance to diseases in several crops including tomatoes. Greenhouse assays have been conducted to evaluate PGPRs, acibenzolar-S-methyl (Actigard 50WG) and silicon (silicic acid) on their potential to enhance plant growth and to suppress diseases of tomatoes such as *tomato yellow leaf curl virus* (TYLCV) and Fusarium crown and root rot. Tomato seeds (cv. FL 47) were sown into pre-mix in 128-cell Styrofoam flats (Speedling, Sun City, FL) and grown for 2 weeks when solutions of PGPR (SE34 and IN937b), Actigard and silicic acid were sprayed on the foliage of the tomato seedlings. By one week after treatment, growth of seedlings treated with PGPR strains SE34 and IN973b had significantly increased compared to those sprayed with the water control ($P = 0.0001$), by an average increase of 27.3% and 17.4% in plant height for SE34 and IN973b treatments, respectively. The chlorophyll content in the leaves from tomato seedlings treated with IN973b was significantly higher (8.6%) compared to the water control ($P = 0.0009$). Seedlings were then transplanted into plastic pots containing potting mix and the experiments are under investigation for the effects of PGPR, Actigard and silicic acid on development of TYLCV and Fusarium crown and root rot. Results from this study will be reported.

Analysis of infectious clones of Oilseed rape mosaic virus (ORMV) in plants

C. ZHANG (1), V. C. Torney (1), C. Yang (1), S. A. Whitham (1)
(1) Iowa State University
Phytopathology 98:S180

Oilseed rape mosaic virus (ORMV or Youcai mosaic virus, YoMV) is a member of the genus tobamovirus of which Tobacco mosaic virus (TMV) is the type member. ORMV is also considered to be a strain of Ribgrass mosaic virus (RMV) due to the very high nucleotide identity shared by these two viruses. ORMV can infect many economically important plants, such as beet, cabbage, turnip, cucumber, pepper, barley, wheat and canola. Infectious clone technology provides an opportunity to study the molecular basis of virus biology and enables the development of viral vectors for gene function study. Here we describe the construction of infectious clones of ORMV and the development of these clones for functional genomics applications. The full-length cDNA of ORMV was produced from reverse-transcriptase polymerase chain reaction (RT-PCR) products of parental viral RNA and placed under control of the CaMV 35S promoter and NOS terminator. A ribozyme sequence was introduced at the 3′ end of ORMV genome for the production of authentic 3′ end genomic RNA and the resulting cDNA clones are highly infectious. Four types of infectious clones were analyzed and whole genomic sequences determined. The results demonstrate the importance of the ORMV infectious clones as functional genomics tools for many important plants.

Two-component signal transduction systems play a major role in *Erwinia amylovora* pathogenesis and survival

Y. ZHAO (1), D. Wang (1), S. Nakka (1)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL 61801
Phytopathology 98:S180

Two-component signal transduction system (TCST), consisting of a histidine kinase (HK) and a response regulator (RR), represents major paradigm for signal transduction in prokaryotes. TCSTs play critical roles in sensing and responding to environmental conditions and in bacterial pathogenesis. On the basis of entire genomic sequence of *Erwinia amylovora* Ea273 from the Sanger Center, and the aid of conserved domains of known HKs and RRs, we conducted a genome-wide identification of TCST genes in the genome of *E. amylovora*. A total of 45 TCST genes in *E. amylovora* including 20 sensor kinases, 19 response regulators, four sensory box proteins, one hybrid and one serine-threonine kinase were identified. We then conducted a systemic TCST gene-knockout experiment and had successfully generated a total of 59 single-, double-, and triple-mutants. Our results showed that five mutants were non-pathogenic on immature pears. Results from phenotypic characterization and gene expression experiments, using GFP as a reporter, of TCST mutants indicate that TCSTs in *E. amylovora* not only control gene expression of hrp-type III secretion system and amylovoran biosynthesis, two major pathogenicity factors in *E. amylovora*, but also regulate gene expression of the two novel T3SS pathogenicity islands (EPI1 and EPI2) and flagella biosynthesis. In addition, we identified both positive (non-motile), negative (hypermotile) and intermediate phenotype regulators for swarming motility in *E. amylovora*. Furthermore, several TCSTs also conferred *E. amylovora* resistance to antimicrobial peptides, iron, and osmotic stress. These results indicate that TCSTs in *E. amylovora* play a major role in its pathogenesis and survival.

Antibacterial activity of endophytic fungi from rhizomes of Paris polyphylla var. yunnanensis

J. Zhao (1), L. ZHOU (1)
(1) China Agricultural University
Phytopathology 98:S180

The crude n-butanol extracts of sixteen endophytic fungi isolated from the rhizomes of Paris polyphylla var. yunnanensis were preliminarily detected for their antibacterial activity by the methods of agar well diffusion assay and a modified broth dilution test. For the further examination of their antibacterial activity and bioassay-guiding separation of these antibacterial compounds, a modified TLC-bioautography method was used. It was found that eight endophytic fungi (i.e. Ppf1, Ppf2, Ppf4, Ppf8, Ppf9, Ppf10, Ppf14 and Ppf15) were screened to show strong antibacterial activity against the test bacteria (i.e. Bacillus subtilis, Staphylococcus haemolyticus, Escherichia coli, Xanthomonas vesicatoria, Agrobacterium tumefaciens and Pseudomonas lachrymans). Minimal inhibitory concentration (MIC) values of the extracts were between 0.0625 and 2.000 mg/ml. These endophytes with better antibacterial activity were identified by morphological characters and internal transcribed spacer (ITS) rRNA gene sequence analysis. The results may provide some evidences for further separation of the antibacterial compounds from these endophytic fungi.

Development and characterization of expressed sequence tag (EST)-derived microsatellite markers for the wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici*

S. ZHONG (1), Y. Leng (1), T. Friesen (2), J. Faris (2), L. Szabo (3)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA; (2) USDA-ARS Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND, USA; (3) USDA-ARS, Cereal Disease Laboratory, University of Minnesota, St. Paul, MN, USA
Phytopathology 98:S180

Puccinia graminis f. sp. *tritici*, the causal agent of stem rust in wheat, has caused devastating disease epidemics throughout history and is still posing a potential threat to wheat production in some regions of the world due to the appearance of new races. To develop microsatellite or simple sequence repeat (SSR) markers for population genetics studies, a total of 60,579 EST sequences (reads) generated from *P. graminis* f. sp. *tritici* were screened for tandemly repeated di- and tri-nucleotide units using a bioinformatics approach, and 708 unisequences containing putative SSR loci with six or more repeat units were identified. Flanking primers were designed for 384 unique SSR loci, which mapped to different locations of the draft genome sequence of the fungus. These primers were tested for amplification and polymorphism by PCR on twenty isolates of *P. graminis* f. sp. *tritici* from North America. Among the 384 primer pairs, 182 failed to produce PCR products, whereas 202 generated amplicons in at least two isolates. Seventy-two of the 202 primer pairs generated easily scored patterns with polymorphism among the isolates tested. Also, 101 primer pairs were found to show polymorphism between the two isolates CRL 78-21-BB463 and CRL 75-36-700-3 of *P. graminis* f. sp. *tritici*, which were used to generate a mapping population. The SSR loci were also amplified in the closely related rye stem rust fungus *P. graminis* f. sp. *secalis*. These SSR markers derived from ESTs will be useful for characterization of population structures and for gene mapping in *P. graminis*.

Hairy vetch-induced systemic resistance to Fusarium wilt in watermelon

X. ZHOU (1), K. L. Everts (2)
(1) University of Maryland, Salisbury, MD; (2) University of Maryland, Salisbury, MD; University of Delaware, Georgetown, DE
Phytopathology 98:S180

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (FON), is the most destructive disease of watermelon worldwide. Hairy vetch (*Vicia villosa*) used as a soil amendment has recently been described as a potential tool for managing the disease, however, the mechanisms of action is not known. In this study, three greenhouse bioassays in which vetch-amended soil was spatially separated from the pathogen were conducted to test whether hairy vetch amendment induced host resistance. In split-root tests, one half of the root systems of wilt-susceptible watermelons (cv. Sugar Baby) were exposed to soil amended with vetch for 3 and 6 days or not amended before the other half of the root systems were challenged with FON in soil. Both 3- and 6-day treatments significantly reduced (over 45%) wilt incidence and colonization

by FON in the lower stems. In stem injection tests, a microconidial suspension of FON was slowly injected into the stems above cotyledons of plants that were grown in soil amended with or without vetch for 7 days. Vetch treatment resulted in a significantly lower wilt severity compared to non vetch treatment. In leaf inoculation tests, when leaves of plants grown in vetch-amended or non-amended soil for 3 weeks were inoculated with *Colletotrichum orbiculare*, lesion number was reduced 46% and lesion area 63% on the leaves of vetch-grown plants compared to the control plants. The results suggest that a soil amended with hairy vetch can induce systemic resistance to Fusarium wilt in watermelon.

Seed transmission of *Candidatus* Liberibacter asiaticus in periwinkle and dodder resulted in low bacterial titer and very mild disease in periwinkle

L. Zhou (2), Y. DUAN (2), D. Gabriel (1), T. R. Gottwald (2)
(1) Dept. of Plant Pathology, University of Florida, Gainesville, FL 32653; (2) USDA, ARS, USHRL, 2001 S. Rock Rd., Fort Pierce, FL
Phytopathology 98:S181

Canadidatus Liberibacter asiaticus (Las) is the most widely-distributed of three species of Liberibacter that are associated with citrus Huanglongbing (HLB), a lethal disease of citrus worldwide. In addition to citrus, periwinkle (*Catharanthus roseus*) and dodder (*Cuscuta pentagona*) are two experimental hosts in which the bacteria can multiply well. Symptoms of HLB in inoculated-periwinkle were characterized by progressive vein and leaf yellowing, resulting in death of most HLB-infected periwinkles within six month after first appearance of symptoms. Dodder plants did not exhibit symptoms, even when they contain high titers of the bacterium. Las was detected in up to 53% of all seeds tested both from HLB-infected periwinkle and dodder without resorting to nested PCR. The PCR amplicons were confirmed by sequence analysis. Germination rates of these Las-positive seeds from both plant species were normal. Over 80% of the periwinkle plants germinated from the infected seeds showed initial HLB symptoms of vein yellowing and leaf yellowing only when they were stressed by nutrient deficiency. Surprisingly, the disease progressed slowly, and did not cause plant death, and all symptomatic plants became asymptomatic after the stress was removed. The Las population remained in very low titer; in most cases, detected only by nested PCR or regular PCR by increasing the concentration of the bacterial DNA. The periwinkles-infected with Las via seed transmission have been maintained for over six months. These results suggest that although Las was seed transmitted, a second, undescribed component of an HLB disease complex was not.