



## Towards indicators of soil health

Céline Janvier, François Villeneuve, Véronique Edel-Hermann, Thierry Mateille, Claude C. Alabouvette, Christian Steinberg

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### Mechanisms involved in spatial and temporal mobility of disease patches caused by *Rhizoctonia solani* in sugar beet field: Induction of antagonists within disease patch

Muhammad Anees, Arne Tronsmo, Véronique Edel-Hermann, Nadine Gautheron, Christian Steinberg .....

1-5

**Abstract:** *Rhizoctonia solani* AG 2-2 causes damping-off and root rot on sugar beet in patches that are highly mobile both on spatial and temporal scales. They never occur in the same place where they were in the previous season. The aim of the present study was to uncover the mechanisms underlying the dynamics of disease patches. It was observed that soil inoculum potential was higher within diseased patch than in healthy area. However, the dormant pathogen in healthy area was stimulated by addition of buckwheat meal more than that in diseased patch. In addition soil from diseased area was more suppressive towards the disease. We did not observe significant differences in bacterial genetic structure with the origin of the soil. However, diseased area could be easily distinguished from healthy area by their bacterial physiological fingerprints. It was observed that *Trichoderma* isolates from diseased patch were more antagonistic than the ones from outside the patch both in vitro and in vivo. These results suggest that *R. solani* when active may stimulate the development of antagonistic organisms. Their presence may inhibit the formation of patches in the subsequent season in that part of the field.

### Take-all decline: An epidemiological analysis

Douglas Bailey, Nik Cuniffe, Philippe Lucas, Marie Gosme, Neil Paveley, John Spink, Christopher Gilligan .....

7-10

**Abstract:** Take-all dynamics within crops differing in cropping history (the number of previous wheat crops) and representing take-all decline, as well as the effects of a chemical seed treatment, were analysed using a simple epidemiological model. The model includes terms for primary infection, secondary infection, inoculum decay, root growth and disease suppression. The net rate of primary infection increased to a maximum after the first wheat crop and was constant thereafter. The rate of secondary infection and its suppression increased to a maximum during the second, third and fourth wheat crops and then decreased as more wheat crops were grown. Chemical seed treatment had no detectable effect on the take-all decline process. We conjecture that these results are consistent with three phases of take-all decline: An initial phase characterized by the build-up of pathogen inoculum and of disease, a second phase characterised by the build-up of antagonists in response to high levels of disease and a final phase characterised by a period of disease suppression.

### *Pythium* elicitors in biological control of *Botrytis cinerea*

Kanak Bala, Dalia Rav David, Bernard Paul, Yigal Elad .....

11-14

**Abstract:** *Botrytis cinerea* is a well-known necrotic pathogen, causing grey mould disease in grapevine and other crop plants, such as tomato and bean. The members of the genus *Pythium* are mostly saprophytes, present in the soil, sometimes acting as parasites, sometimes as effective mycoparasites. There are reports that they secrete proteins like oligandrin (*P. oligandrum*) that plays a vital role in the enhancement of systemic resistance in the plants. In this paper, we present the active involvement of substances secreted by *P. hypogynum* and *P. canariense* in providing control against *B. cinerea* to tomato and bean plants. Filtrates from *Pythium* spp., when applied either on the leaves or on the roots, prior to exposure to *B. cinerea*, reduced conidial germination on leaves, reduced disease severity and conferred disease resistance in tomato.

## Population dynamics of beneficial microorganisms co-applied to seed during drum priming

*Amanda J. Bennett, John M. Whipps* ..... 15-18

**Abstract:** This work showed for the first time that combinations of one fungal and one bacterial isolate could be successfully applied to seed during the commercially realistic process of drum priming. Beneficial microorganisms included the fungi *Clonostachys rosea* IK726d11 and *Trichoderma harzianum* T22 and the bacteria *Pseudomonas chlororaphis* MA342 and *Pseudomonas fluorescens* CHA0. All combinations tested were compatible with each other on application to carrot and onion seed, and all microorganisms survived above the target of 5 log<sub>10</sub> cfu g<sup>-1</sup> dry seed at the end of the priming process. Glasshouse experiments showed that the population dynamics of the fungi and bacteria on the roots and in the rhizosphere soil once the seed was planted were similar whether the microorganisms were applied singly or in combination. However, the effects of the microorganism combinations on seedling emergence and growth varied depending on the host plant and which isolates were present.

## Microbial analysis of soils from avocado crops modified by different organic amendments and its role in disease suppressiveness

*Nuria Bonilla, Juan A. Torés, Alejandro Pérez-García, José M. Hermoso, Jorge Gonzalez, David Sarmiento, Francisco M. Cazorla, Antonio de Vicente* ..... 19-22

**Abstract:** In southern Spain, the most destructive root rot diseases of avocado are caused by *Rosellinia necatrix* and *Phytophthora cinnamomi*. The addition of organic amendments or mulches produced some benefits; among them, seem to prevent the disease by their influence in the microbial balance in the soil. In this sense, we are studying the effect of the application of different types of amendments to avocado trees, in order to get insight about their influence on the soil microbial communities and activities. The analysis of cultured microorganisms revealed the importance of specific groups of microorganisms in these soils, like actinomycetes or the genera *Pseudomonas*. The culture-independent technique DGGE revealed the presence of complex bacterial communities in the different treatment with organic mulches.

## Diversity of *Sclerotinia sclerotiorum* in the UK and variability in germination of sclerotia

*John Clarkson, Emily Clewes, John Whipps* ..... 23-26

**Abstract:** *Sclerotinia sclerotiorum* isolates were collected from carrot, lettuce, and oilseed rape crops from different locations more than 100 km apart in the UK. Isolates were characterised by assigning them to mycelial compatibility groups (MCGs) and also by sequencing the internal transcribed spacer (ITS) and a section of the intergenic spacer (IGS) regions of the ribosomal RNA (rRNA) gene repeat, as well as part of the elongation factor 1 alpha gene (EF1- $\alpha$ ). MCG tests showed diversity within the 96 isolates tested (32 from each crop), with a group of 13 compatible isolates being the most frequently found MCG in each crop (5 each from carrot and lettuce and 3 from oilseed rape). A further 64 MCGs comprised 1-3 isolates. Sequencing revealed that the *S. sclerotiorum* isolates could be separated into two, three and six groups respectively for ITS, EF1- $\alpha$  and IGS. Compatible isolates were generally in the same group. The ability of sclerotia from 46 *S. sclerotiorum* isolates to germinate and produce apothecia was also assessed under controlled conditions and a wide range of germination times and numbers of apothecia produced was observed.

## Towards understanding the temporal dynamics of *Allium* white rot: factors affecting the infectivity of *Sclerotium cepivorum* sclerotia

*John Clarkson, Anita Scruby, John Whipps* ..... 27-30

**Abstract:** *Sclerotium cepivorum* causing *Allium* white rot (AWR) is a major pathogen of onion and garlic crops worldwide. The effect of soil temperature and other factors on the ability of sclerotia to survive and overcome dormancy and germinate was investigated. Laboratory experiments showed that germination of sclerotia in response to the artificial germination stimulant diallyl disulphide (DADS) was influenced both by the substrate on which they were formed and also by drying. Sclerotia conditioned in soil between 5 and 30°C in order to break dormancy had high germination levels after just 2 weeks compared with those which were unconditioned. However, a decline in sclerotial survival was evident at 30°C. Sclerotia with a high germination response to DADS also caused more AWR on onion plants in the glasshouse compared with those with poor germination, demonstrating that the assay was a good indicator of

infectivity. In field experiments, sclerotia buried in October, January and May all exhibited increasing potential to germinate with time with some evidence to suggest that this decreased after extended periods. Survival of sclerotia varied between burials, but viable sclerotia were still detected after one year.

#### Antagonistic ability of *Bacillus subtilis* strains against *Fusarium oxysporum* f.sp. *radicis lycopersici*

Constantinescu Florica, Voshol G.P., Validov Shamil, Bloemberg Guido ..... 31-35

**Abstract:** The excessive use of chemical pesticides has caused soil pollution, phytopathogen resistance and harmful effects on flora, fauna and humans. Therefore, biological control of soil borne diseases by using microbial pesticides that suppress plant pathogens is an attractive tool for horti- and agriculture. *Fusarium oxysporum* f. sp. *radicis-lycopersici* occurs frequently in greenhouse conditions (mainly at 25-27°C and 80-100% soil humidity) affecting tomato seedlings and causing foot and root rot. Four *Bacillus subtilis* strains, B49b, Bce2, B30 and MB2 were selected for antagonistic activity against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and analyzed for their biocontrol ability. *In vitro* tests showed that all the tested strains produced antifungal metabolites, as was shown by growth inhibition zones. The strongest inhibition was caused by *B. subtilis* B49b and Bce2 followed by B30 and MB2. Greenhouse experiments showed that all *Bacillus* strains significantly reduced disease symptoms, and that the highest protection of tomato plants was conferred by strains B49b and Bce2. In order to identify the antifungal metabolites produced by these strains, four liquid media were tested and three different solvents were used to extract the spent culture supernatant. *In vitro* testing of the crude extracts obtained with 3 different solvents showed that the highest inhibition zone was induced by the ones extracted with ethyl acetate + 1% formic acid. Future studies will focus on the purification and identification of the antifungal compounds and their role in biocontrol activity.

#### Biodiversity and soil quality in agroecosystems: the use of a qualitative multi-attribute model

Jérôme Cortet, Marko Bohanec, Bryan Griffiths, Martin Žnidaršič, Marko

Debeljak, Sandra Caul, Jacqueline Thompson, Paul H. Krogh ..... 37-40

**Abstract** In ecological impact assessment, special emphasis is put on soil biology and estimating soil quality from the observed biological parameters. The aim of this study is to propose a tool easy to use for scientists and decision makers for agroecosystems soil quality assessment using these biological parameters. This tool was developed as a collaboration between ECOGEN (www.ecogen.dk) soil experts and decision analysts. Methodologically, we have addressed this goal using model-based Decision Support Systems (DSS), taking the approach of qualitative multi-attribute modelling. The approach is based on developing various hierarchical multi-attribute models that consist of qualitative attributes and utility (aggregation) functions, represented by decision rules. The assessment of soil quality is based on two main indicators: (1) soil diversity (assessed through microfauna, mesofauna and macrofauna richness) and (2) soil functioning (in terms of leaching, comminution, mineralisation and plant growth). Here we address the methodological aspects of the model and its development, and describe the components of the model (attributes, value scales, decision rules and weights). We present initial results of its application to the assessment of crop management on soil quality in the case of using conventional and *Bt*-maize at three field sites in France and Denmark. Our finding was that soil quality was unaffected even though several input attributes were changed. We also analyse the sensitivity of the model and discuss its current and potential contribution to soil quality management.

#### Real-time PCR assay for identification and quantification of *Rhizoctonia solani* AG-2-2 in soil

Véronique Edel-Hermann, Marlène Jobard, Nadine Gautheron, Hanna Friberg,

Christian Steinberg ..... 41-46

**Abstract:** *Rhizoctonia solani* AG-2-2 causes severe damages on sugar beet, which appear in the field as dispersed and unpredictable patches of diseased plants. Knowledge of the ecology of this pathogen is limited by the absence of diagnostic tool. We have developed a specific real-time PCR assay enabling the identification of *R. solani* AG-2-2 and its direct quantification in soil.

## Effect of organic matter on soil inoculum potential and soil suppressiveness to

*Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia solani*

Léon Fayolle, Claude Alabouvette, J.M. Bodet, Christian Steinberg ..... 47-51

**Abstract:** It is widely recognized that organic amendments may reduce crop losses caused by soil borne plant pathogens. Most of the studies conducted so far were related to short term effects observed in vegetable cropping systems. The long term effects of compost or manure amendments in field cropping systems has been investigated in Australia and, concerning *Rhizoctonia solani* diseases on wheat, inconsistent results were reported. Two large field experiments were set up in 1996, at Le Rheu and la Jaillière (France), to study the effects of organic fertilization in wheat and maize production. In 2006, at the end of the experiments, soil was sampled to assess the effects of the organic matter fertilization on the soil inoculum potential and soil suppressiveness to diseases caused by *Gaeumannomyces graminis* var. *tritici* (Ggt, take all) and *R. solani*. Bioassays were set up in growth chamber, using wheat and carrots to measure soil inoculum potential and soil suppressiveness to Ggt and *R. solani*, respectively. A pathological index was used to evaluate the disease severity caused by Ggt on wheat and area under the disease progress curve (AUDPC) was calculated to quantify the disease severity caused by *R. solani*. ANOVA was used to compare the data. At both locations, the inoculum potential of the soils naturally infested by Ggt was low and similar in plots amended with different organic matters and mineral fertilization. The level of soil suppressiveness to take-all was high in plots amended with organic matter compared to the low level after mineral fertilisation. This suggests that organic amendments preserved the suppressive ability of the soil towards take all when a wheat/maize rotation is performed. Concerning *R. solani*, the soil inoculum potential was reduced in plots amended with organic matters, but only at La Jaillière not at Le Rheu. At both 2 locations, soil suppressiveness to *R. solani* damping-off was not affected by organic amendments. There were neither clear positive nor detrimental effects of organic fertilisation in a wheat/maize rotation. Therefore organic matter can be used to improve soil fertility without any negative effects on the phytosanitary status of soil.

## Identification of a fungal population associated with soil suppressiveness to

*Rhizoctonia solani* diseases in a biofumigated soil

Marie Fiers, Céline Janvier, Christian Steinberg, Véronique Edel-Hermann,

François Villeneuve, Claude Alabouvette ..... 53-56

**Abstract:** Biofumigation was applied before the culture of carrots for two years on an experimental plot. Assessment of soil suppressiveness to *Rhizoctonia solani* damping-off showed that biofumigation increases the level of soil suppressiveness in comparison to the untreated control. Microbial analyses were performed on soil samples collected from each plot to compare both the density and the structure of the microbial communities. The fungal population density was higher in the biofumigated plot; moreover one fungal genus, *Aspergillus*, was dominant. Cloning and sequencing of the ITS region of the dominant *Aspergillus* colonies enabled us to identify the strains as *A. fumigatus* with 97 to 100 % of similarity. Terminal restriction fragment length polymorphism (T-RFLP) analysis applied to total DNA extracted from soil revealed a significant difference in the structure of the fungal communities between the biofumigated plot and the control. This difference was based on the presence of a few discriminating terminal restriction fragments (TRF). T-RFLP analyses applied to isolates of *A. fumigatus* enabled us to determine the TRF of this species, which fits perfectly with one of those revealed by T-RFLP analysis from soil DNA. Using both traditional microbiological techniques (soil plate dilution) and molecular tools enabled us to detect and identify one fungal population which is a marker of the biofumigation. Whether *A. fumigatus* is just a marker of biofumigation or is involved in the mechanisms controlling soil suppressiveness has still to be investigated.

## Interactions between bacteria and ectomycorrhizal fungi: what's new?

Pascale Frey-Klett, Aurélie Deveau, Mika Tarkka, Stéphane Uroz, Béatrice Palin,

Jean-Claude Pierrat, Francis Martin, Jean Garbaye ..... 57-61

**Abstract:** The tree-soil interface in boreal and temperate forest ecosystems consists of a diverse community of ectomycorrhizal short roots closely associated with bacteria. This multitrophic ectomycorrhizal complex plays a central role in gross production and nutrient cycling. So far, its functioning has been poorly documented. However, recent studies have stressed the importance of physical, metabolic and functional interactions between bacteria and ectomycorrhizal mycelia.

Many mycorrhizosphere bacteria live in contact with ectomycorrhizal fungi, forming biofilms on the hyphal surface or even colonizing the intracellular compartment. Surprisingly, whereas bacterial endosymbioses are diverse and widespread in the plant and animal kingdoms, they have very rarely been reported for fungi, except within the Glomeromycota phylum. The occurrence, diversity and ecology of such intracellular bacteria will be illustrated here in the case of the ectomycorrhizal fungus *Laccaria bicolor*, commercially used in France to enhance the growth of Douglas fir plantations. In the case of the so-called Mycorrhiza Helper Bacteria (MHBs), which promote the establishment of the ectomycorrhizal symbiosis, different mechanisms underlying this effect have been proposed. Recent advances in the metabolic and molecular analyses of the interactions between helper bacteria and ectomycorrhizal fungi will be presented.

**Response of *Rhizoctonia solani* to soil faunal grazing and organic amendments – different from general microbial dynamics**

*Hanna Friberg, Léon Fayolle, Véronique Edel-Hermann, Nadine Gautheron,*

*Céline Faivre Christian Steinberg* ..... 63-67

**Abstract:** In a four-month microcosm experiment, we studied the effect of enchytraeids and earthworms on dynamics of *Rhizoctonia solani* in soil amended with different types of organic material. Special focus was on comparing the effects with those on total microbial densities, to see if the development of the pathogen follows the general dynamics of saprophytic bacteria and fungi in the system. We found that animal effects on the density of *R. solani* and on the densities of culturable fungi and bacteria were dependent not only on type of animal present, but also on the amount and type of organic material available. The highest densities of bacteria and fungi were found in soil amended with fresh organic material in presence of earthworms and the lowest densities in non-amended soil with enchytraeids. While the bacteria and fungi responded similarly to animal and organic material treatments, the response of *R. solani* showed an opposite pattern with the highest densities observed in the treatments with the lowest densities of fungi and bacteria. These results show that *R. solani* does not follow the general dynamics of soil microorganisms. As the highest densities of *R. solani* were found in treatments with a high grazing pressure, our findings also indicate that *R. solani* may get a competitive advantage from soil faunal grazing.

**Synergy of *Brassica napus* green manure and *Trichoderma* seed treatment against *Sclerotium rolfsii* of sugar beet**

*Stefania Galletti, Pier Luigi Burzi, Eleonora Sala, Simona Marinello, Claudio*

*Cerato* ..... 69-72

**Abstract:** *Sclerotium rolfsii* Sacc., agent of sugar beet root rot, causes severe economical losses in several sugar beet areas, where high temperatures involve frequent watering, thus providing optimal conditions for the disease. As for other soilborne pathogens, the adoption of correct agronomic practices can only prevent the damage. Under favourable conditions in fact the disease rapidly spreads without possibilities of control, even by chemicals. Thus a preventive biological approach has been studied which integrates different strategies, with the aim of lowering the pathogen inoculum into the soil and protecting the roots, while safeguarding the environment. The approach studied was based on the combination of the green manure of a biocidal crop (*Brassica napus* L.) with the treatment of sugar beet seeds by a biological control agent (*Trichoderma* spp.) previously selected for *in vitro* antagonism towards *S. rolfsii* and competence for sugar beet rhizosphere. The experiment was carried out in central Italy in a highly infested field according to a split-plot design with 2 replicates and 2 factors: *B. napus* amended/unamended soil on main plots; *Trichoderma* treated/untreated sugar beet seeds on sub-plots. The effectiveness of the different treatments was evaluated as the number of healthy sugar beet plants per m<sup>2</sup> at harvest. The green manure of the biocidal crop in combination with the seed treatment with selected *Trichoderma* strains, gave significant differences from the single measures alone, suggesting the existence of a kind of synergy between the two methods of biocontrol. These findings would seem to open new perspectives for reducing the effects of this chemically uncontrollable disease of sugar beet.

## Contribution of bacteria to the functional diversity of ectomycorrhizas in forest ecosystems

**Jean Garbaye** ..... 73-76

**Abstract:** Ectomycorrhizal communities are taxonomically and functionally diverse, and their functional diversity is partly due to multitrophic interactions with more or less specialized bacteria that are closely associated to the symbiotic tissues of the ectomycorrhizas and cooperate with the fungal partner. This paper briefly reviews research results demonstrating the significant contribution of ectomycorrhiza-associated bacteria in the case of three root functions: nitrogen fixation, mineral weathering and protection against root pathogens.

## Differentially expressed genes during interactions in a fungistatic bacterial mixture

**Paolina Garbeva and Wietse de Boer** ..... 77-81

**Abstract:** Mixing of different bacterial species with little or no apparent antagonism against fungi can result in strong fungal suppression (De Boer *et al.*, 2007). Here we show that direct contact between the bacterial species is not essential to obtain fungal suppression. To elucidate the mechanism of the triggering of fungal suppression by interspecific bacterial interactions, we analyzed genes that are expressed in mixed cultures but not in monocultures. We studied this differential gene expression using Random Arbitrary Primed-PCR. The genes expressed during interspecific bacterial interactions fell in the following categories: Unknown function, catabolic function, regulatory function, chemotaxis function, and possible secondary metabolite production.

## Induction of systemic resistance by PGPR, a suitable means to consider for managing of cucurbit powdery mildew

**Laura García-Gutiérrez, Diego Romero, Houda Zeriouh, Francisco M. Cazorla, Antonio de Vicente, Alejandro Pérez-García** ..... 83-86

**Abstract:** *Podosphaera fusca* is the causal agent of cucurbit powdery mildew in Spain. Fungicide applications and the use of resistant cultivars represent the main means of control. However, outstanding limitations to their effectiveness are favouring the progress of biological control approaches and their integration into feasible integrated pest management programs. In this study we described a screening for plant growth-promoting rhizobacteria (PGPR) in cucurbits and the selection of two *Pseudomonas* spp. and three *Bacillus* spp. strains which applied to roots of melon plants were able to promote 45% increase of fresh weight and reduction of disease severity up to 85% by induction of systemic resistance (ISR).

## Ecophysiological Influence of TerralytPlus® on electrochemical soil parameters

**Hartmut Heilmann** ..... 87-90

**Abstract:** Practical experiences in on-farm-research confirm the model of an organic autoregulation of pH values in the soil. The testing agent Terralyt Plus® as a soil electrolyte has influence on microbial processes. Investigations on the role of the electrochemical tension between Cu- and Al-electrode (Bertrammeter values) support the model of a chemical soil activity influenced by a reducing path originating in the plants.

## Trophic interactions between soil fungi and Collembola

**Gloria Innocenti, Maria Agnese Sabatini, Sonia Ganassi, Matteo Montanari, Maria Barbara Branzanti** ..... 91-94

**Abstract:** Results of our studies on interactions between different species of soil fungi and collembola *in vitro*, and *in planta* are summarised. The feeding preference of collembolan *Protaphorura armata*, *Mesaphorura krausbaueri*, *Onychiurus tuberculatus*, and *Folsomia candida* were investigated in Petri dishes, each containing contemporaneously *Fusarium culmorum* (Fc), *Gaeumannomyces graminis* var. *tritici* (Ggt), *Bipolaris sorokiniana* (Bs) and *Rhizotonia cerealis* (Rc) colonies. In some experiments Bs was replaced by *Trichoderma harzianum* (Th). All springtails grazed preferably on the mycelium of Fc, but Ggt and Rc hyphae were also consumed. Bs mycelium had repellent and/or lethal effect on animals, Th mycelium was repellent, whereas conidia of both fungi were palatable. A diet based on Fc, Ggt, and Rc mycelium or Bs conidia showed different impact on the life processes of *M. krausbaueri*. The fate of conidia during gut passage was also considered. The majority of Th conidia were not damaged during transit through the gut, and thus they were viable and formed colonies. On the contrary, transit through the gut damaged the conidia of Fc. *In planta* studies have shown that *P. armata* significantly controlled disease caused by Fc or Ggt on wheat seedlings. The compatibility between Th and springtails in the presence of Ggt was also investigated in *in planta* assays. It has

been observed that when *Th* propagules coated wheat seeds, springtails showed biocontrol effect against *Ggt* disease. On the contrary, when *Th* inoculum was mixed with the substrate of plant growth, the animals were not able to control disease severity. In all experiments the biomass of wheat plants was not reduced by springtails.

#### Towards indicators of soil health

*Céline Janvier, François Villeneuve, Véronique Edel-Hermann, Thierry Mateille, Claude Alabouvette, Christian Steinberg* ..... 95-100

**Abstract:** Soil is a finite and dynamic living resource. Soil health arises from multiple interactions between physicochemical and biological components, including microbial communities, of primary importance for soil functioning. Facing the threat of soilborne pathogens, cultural practices, as "ecological" crop protection methods, are more and more used. Their aim is to modify the soil microbial equilibrium. In order to measure soil health and to propose cultural practices to improve it, it is necessary to define indicators of soil health. The aim of this study is to propose indicators of soil health, through the evaluation of the impact of two cultural practices (amendment with composted cattle manure and biofumigation) on physicochemical and biological characteristics of the soil, in relation with the phytosanitary quality of a carrot crop. Multivariate analyses, associated with co-inertia analyses, revealed that some descriptors of the soil could be considered as potential indicators of soil health. In particular, these descriptors are associated with biofumigation, which enhance the soil suppressiveness to *Rhizoctonia solani* diseases. Descriptors could then be integrated in a minimum data set of indicators of soil health. This study could be continued by the confirmation of the interest of these descriptors, the construction of an indicator, and its validation.

#### Spatial pattern and temporal dynamics of *Sclerotium* root rot (*Sclerotium rolfsii*) in fall sown sugar beet crops in southern Spain

*Rafael Jordán-Ramírez, Rafael M. Jiménez-Díaz, Juan A. Navas-Cortés* ..... 101-103

**Abstract:** The spatial pattern and temporal dynamics of *Sclerotium* root rot, caused by *Sclerotium rolfsii*, was investigated in five sugarbeet plots located in southern Spain during two consecutive crop seasons. Ordinary runs analysis generally indicated a departure from randomness of diseased plants status adjacent plants within rows. The binomial index of dispersion and the interclass correlation and estimates parameters for the binary form of Taylor's power law for the various quadrat sizes suggested aggregation of symptomatic plants for all plots. Aggregation in each plot appeared to be related with disease incidence. Spatial analysis by distance indices showed a non-random arrangement of quadrats with infected plants. Spatial pattern was often characterized by the occurrence of several clusters of infected plants. Increasing clustering over time was evidenced by stronger values of clustering index and increasing patch cluster size in successive disease assessment dates. Strong significant spatial associations were detected between the number and size of the spatial aggregates during consecutive time periods in each season, indicating the occurrence of secondary cycles of the pathogen. Sugar beet root yield and sugar content was spatially and negatively correlated with SRR incidence and severity. On the contrary, non-sugar compounds (i.e., Na, P, alpha-amino N) were positively correlated with both disease components. High levels of these compounds have been related to plant stress, indicating a detrimental effect of SRR in both root yield and industrial quality of SRR infected sugar-beet roots.

#### Biocontrol activity of *Collimonas fungivorans* against tomato foot and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*

*Faina Kamilova, Johan Leveau, Ben Lugtenberg* ..... 105-108

**Abstract:** Although bacteria belonging to genus *Collimonas* have shown *in vitro* antifungal activity against many different fungi, they appeared inactive against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl), the causal agent of tomato foot and root rot (TFRR). Visualization studies using fluorescently labeled organisms showed that bacterial cells attached extensively to the fungal hyphae under nutrient-poor conditions but not in glucose-rich Armstrong medium. *Collimonas fungivorans* was shown to be as efficient in colonizing tomato root tips as the excellent colonizer *Pseudomonas fluorescens* strain WCS365. Furthermore, it appeared to colonize the same sites on the root as did Forl. Under greenhouse conditions in potting soil, *C. fungivorans* performed as well in biocontrol of TFRR as the well-established biocontrol strains *P. fluorescens* WCS365 and *P. chlororaphis* PCL1391. Moreover, under biocontrol conditions, *C.*

*fungivorans* did not attach to Forl hyphae colonizing plant roots. Based on these observations, we hypothesize that *C. fungivorans* controls TFRR mainly through the mechanism of competition for nutrients and niches rather than through its reported mycophagous properties, for which attachment of the bacteria to the fungal hyphae is assumed to be important.

#### Implications of root spatial relationships in young wheat obtained from CT-scanning for an invasion by fungal pathogens

Adam Kleczkowski, Douglas J. Bailey, Wilfred Otten, Margaret Grose,

Christopher A. Gilligan ..... 109-112

**Abstract:** We analyse the way in which spatial arrangement of roots of plants provides a landscape for spread of soil microorganisms. We use a pathozone concept to characterise the behaviour at the individual root level, whereas percolation theory is used to scale-up to the population of roots. Sectional images of living wheat roots were obtained non-destructively by whole-body computed tomography X-ray scanning (X-ray CT). The data were subsequently interpreted in the light of a potential for spread of a fungal pathogen, initiated from a soil propagule and subsequently realized through a root-to-root transmission. We show that realistic root systems can support very different potential for microorganisms spread, with rapid switches from non-invasive to invasive behaviour. The switch can be controlled by time or nutrition (increase in root density resulting in invasion) or properties of the pathogen or interactions with other microorganisms (increase in pathozone width resulting in invasion). There is a substantial variability among plants so that the depth of a zone of potential spread can significantly differ even for plants growing under very similar conditions.

#### Phylogenetic analysis of endophytic *Fusarium oxysporum* strains inducing systemic resistance against the burrowing nematode *Radopholus similis* in banana

Andreas Kurtz, Alexander Schouten and Richard A. Sikora ..... 113-118

**Abstract:** The *in vivo* antagonistic potential of non-pathogenic *Fusarium oxysporum* towards plant pathogenic *F. oxysporum* is well documented. Recently, it was also demonstrated that specific endophytic non-pathogenic *F. oxysporum* strains can successfully reduce the colonization of banana roots by the burrowing nematode *Radopholus similis* (Vu *et al.*, 2004). This nematode is a destructive pest in banana-plantations, causing severe losses world wide. Phylogenetic analysis by means of restriction fragment length polymorphism (RFLP) of the ribosomal intergenic spacer (IGS) region was initiated to compare these isolates not only with each other, but also with pathogenic isolates of *F. oxysporum* f. sp. *cubense* (*Foc*) and with non-antagonistic isolates. This showed that the antagonistic isolates were more related to each other, even when originating from different geographical regions. Remarkably, also *Foc* isolates clustered with one of the biocontrol isolates. Unfortunately, the polymorphism obtained by this analysis was relatively low. To improve the phylogenetic analysis, we sequenced the IGS region of the different strains. In this way a higher polymorphism was identified, leading to a more rigid phylogenetic tree, in which other IGS sequences present in the NCBI database were included as well. The results of this sequence analysis are discussed, together with their use to develop a screening system for promising *F. oxysporum* biocontrol isolates from other crops and banana cultivars against the colonization by nematodes.

#### Influence of Disease Resistant Management Strategies on Genetic and Pathogenic Diversity in Plant Pathogen Populations: *Fusarium* wilt of chickpea, a case study

Blanca B. Landa, M. Mar Jiménez-Gasco, and Rafael M. Jiménez-Díaz ..... 119-122

**Abstract:** *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) is managed primarily by the use of resistant cultivars. However, the efficiency of this disease management strategy may be limited by pathogenic diversity existing in *Foc* populations in soil. In 1992, a field plot without a history of chickpea in Córdoba, southern Spain, was artificially infested with a single *Foc*-race 5 genotype. Then, this field was continuously used for race 5-resistance screening of chickpea germplasm. In 1997, disease developed in race 5-resistant cv. PV1 which is susceptible to *Foc* races 0, 1A, 1B/C, and 6. Affected plants showed a yellowing syndrome that is induced only by races 0 and 1B/C. In 1998, 2001, 2002, 2004 and 2005, a large collection of *F. oxysporum* isolates (over 600) from stems of different chickpea genotypes were typed into *formae specialis ciceris* and *Foc* races by means of specific-PCR assays and genetic fingerprinting analyses. Results indicate the development of *Foc* race 0, *Foc* race -6, or other *Foc* unidentified race, in the *Foc*-race 5 infested field plot following continuous use of it for race 5-resistance

screening. Because we consider unlikely that those races (0, 6, 2 unidentified) had been introduced into the plot, our results suggest that chickpea monoculture in the infested soil may have favoured changes in the virulence and/or pathogenicity structure in *Foc* and/or *F. oxysporum* populations that initially inhabited the soil.

#### Compost performances in a turf system

**Matteo Montanari , Gloria Innocenti, Sante Scagliarini..... 123-126**

**Abstract:** a field experiment was carried during 2004-2006, to assess benefits of utilising compost amended or not with Ca-Lignosulphonate, a lignin-like by-product, in a turf system settled in a ruderal area. Agronomic parameters (green mass yield, leaf length, and chlorophyll content), microbial parameters (total microbial activity, and fungal and bacterial population densities), and physico-chemical parameters (pH, Ec, aggregate stability and water infiltration) were determined. Results obtained show that lignosulphonate addition improves compost agronomic performances associated with higher microbial activity, higher fungal density and better soil physical qualities compared to those of not enriched compost

#### Transformation of *Gnomonia fragariae*, the cause of strawberry root rot and petiole blight, with GFP gene and the study of host infection and colonization

**Inga Moročko, Jamshid Fatehi..... 127-130**

**Abstract:** *Gnomonia fragariae* is a poorly studied ascomycete, which was recently demonstrated to be a cause of severe root rot and petiole blight of strawberry. The pathogen was genetically transformed with the green fluorescent protein (GFP) gene as a vital marker and hygromycin resistance gene. Several stable transformants were obtained, which did not differ in their colony morphology, growth rate, sporulation and pathogenicity from the wild type isolate. One of the transformants was further used for the inoculation of strawberry plants in order to study the infection process and colonization of roots and petioles of the host plant by the pathogen. The present work showed that the use of GFP as reporter was a useful and informative approach to study the infection and colonization pattern of strawberry by *G. fragariae*.

#### Interconnectivity of habitats in soil: combining X-ray micro tomography and thin sectioning to reveal fungal-soil structure interactions

**Wilfred Otten, Kirsty Binnie, Iain Young, Jonathan Villot, Dmitri Grinev ..... 131-134**

**Abstract:** The extreme heterogeneity and interconnectivity of the 3-dimensional pore space within soil makes it a unique habitat for the diverse microbial population and has a pivotal role in microbial interactions. Manipulation and quantification of the 3-dimensional pore space and the spatial distribution of micro-organisms is therefore essential if we are to fully understand microbial interactions. Here we pack soil microcosms at different bulk-densities to manipulate soil structure and use x-ray micro tomography and soil thin sections to analyse the effect on the connectivity of the pore volume and on fungal exploration.

#### Effect of *Glomus mosseae* BEG12, *Trichoderma viride* TV1 and *T. harzianum* T39 on plant growth of healthy and *Armillaria mellea* inoculated strawberry plants under greenhouse controlled conditions

**Ilaria Pertot, Federica De Luca ..... 135-138**

**Abstract:** *Armillaria mellea* causes root rot on numerous plant species. *Glomus mosseae* BEG12, *Trichoderma viride* TV1 and *T. harzianum* T39 can increase grapevine plant growth, both in field and greenhouse, with growth enhancement strongly related to environmental conditions. The aim of this research was to test the effect of *G. mosseae* BEG12, *T. viride* TV1 and *T. harzianum* T39 on healthy and *A. mellea* artificially inoculated strawberry plants under greenhouse controlled conditions. Three replicates of strawberry potted plants (cv. Elsanta) were used in each treatment. Healthy plants and plants inoculated with *A. mellea* infected wood pieces were treated with the three microorganisms. Untreated healthy and *A. mellea* inoculated plants were used as controls. Total leaf surface and number of leaves were weekly assessed. *T. harzianum* T39 and *G. mosseae* BEG12 treatments were able to prevent *A. mellea* infections until the end of the experiment (16 weeks), but they did not kill the pathogen, which was still alive inside the infected wood. *T. viride* TV1 was not able to prevent *A. mellea* infections on strawberry. Both on healthy and inoculated plants, *T. harzianum* T39 induced a temporary positive effect on total leaf surface, which disappeared in the second part of the experiment. *T. viride* TV1 showed a similar initial positive trend, even if not statistically significant compared to the untreated healthy plants, whereas the total leaf surface of treated infected plants heavily decreased after 64 days compared to the treated healthy control,

similarly to the untreated *A. mellea* inoculated plants. *G. mosseae* treatment did not show differences between healthy and inoculated plants, but after 10 weeks the total leaf area was significantly higher compared to the untreated healthy control and leaves were apparently greener. Leaves of untreated *A. mellea* infected strawberries quickly wilt, and then plants reacted by producing new ones, but smaller and yellow.

#### Agricultural practices affect microbial functional diversity, microbial activity and suppressiveness against soil-borne diseases

Michael Raviv, Ido Aviani, Yael Laor, Anat Yogev, Ibrahim Saadi, Rony Cohen, Shlomit Medina ..... 139-144

**Abstract:** The effects of organic vs. conventional fertilisation practices on microbial functional diversity, total microbial counts, biological activity and soil suppressiveness against a representative soil-borne disease were studied. The study site was an experimental organic plum orchard, located in Jezre'el Valley, Israel. A control treatment (trt.) was fertilised conventionally, according to commercial practices, while all plant protection practices were handled according to organic practices. The organic trt. was fertilised with compost and feather meal. The total applied N was similar in both treatments, at  $\sim 350 \text{ Kg ha}^{-1} \text{ year}^{-1}$ . Treatments were compared for their general microbial activity (counts, fluorescein diacetate hydrolysis, heat generation as measured using calorimetry) and for functional diversity using Biolog Eco plates®. Biolog plates were inoculated with increasing soil dilutions ( $10^3$ ,  $10^4$ ,  $10^5$ ) and colour development was recorded. Treatments were compared by richness and Shannon index. Suppressiveness of the soils was assessed using the pathosystem melon (*Cucumis melo* cv. Ofir) and *F. oxysporum* f. sp. *melonis*. Microbial activity was higher in the organic trt. as compared to the control in all tested parameters. Functional diversity of the  $10^5$  but not of lower dilutions was significantly higher in the organic treatment (trt.) both in terms of richness and Shannon index. The organic trt. suppressed wilting of fusarium-inoculated melon plants. It is concluded that substituting chemical fertilisers with compost facilitated the development of healthier soil.

#### Consequences of liming on ectomycorrhizal community structure and functioning

François Rineau, Jean Garbaye ..... 145-150

**Abstract:** Liming is a forestry practice consisting in direct CaO and MgO amendment on the surface of the soil, used to correct tree magnesium and calcium deficiency induced by soil acidity. Soil processes are strongly affected by these calcareous amendments, and ectomycorrhizal (ECM) community structure and functioning is closely linked to soil nutrient availability. The aim of this study was to measure the impact of liming on ECM population structure and ability to mobilize nutrients from soil organic matter. Enzyme assays were done *in vivo*, with excised root tips, in microplates, and using fluorescent or coloured substrates. Results showed i) drastic changes in ectomycorrhizal community structure in limed plots, with apparition of new dominant and neutrophilic morphotypes and changes in vertical distribution in ECM abundances; ii) increased lignocellulolytic enzyme production in spruce ECMs and opposite response in beech ECMs; iii) significant morphotype and tree species effect for all enzymatic activities, and significant liming and soil layer effect on some lignocellulolytic enzyme activities. Finally, liming changes ECM community structure by promoting new neutrophilic species in the organomineral layer, shifting the ability of the ECM community to degrade organic matter to more (spruce) or less (beech) lignocellulolytic enzymatic activities.

#### Interaction between *Brassica carinata* seed meal and *Trichoderma* in soil infected by *Pythium ultimum*

Eleonora Sala, Stefania Galletti, Pier Luigi Burzi, Claudio Cerato ..... 151-154

**Abstract:** Soil amending with *Brassicaceae* seed meals is an attractive alternative to the green manure to achieve a natural fumigation effect based on the glucosinolate-myrosinase system which releases hydrolysis products active against several plant pathogens. One of the problems encountered after *Brassicaceae* meal incorporation into the soil is a rebound effect towards the pathogen population observed after a first decline, probably due to the enrichment in organic substrate. The aim of this study was to find a biological control agent suitable to be combined with the seed meal amending, also in order to compete with the pathogen. A previous screening among 40 *Trichoderma* isolates led to the identification of an isolate tolerant to biocidal compounds released by commercial *Brassica carinata* meal, able to readily colonise such meal and antagonist towards soil-borne pathogens. Such isolate was utilized in combination with *B.*

*carinata* meal in preliminary trials carried out in trays filled with natural soil inoculated with a pathogenic isolate of *Pythium ultimum*, under controlled conditions. The pathogen population in soil decreased immediately after the meal treatment but in the time course tend to increase up to values higher than the untreated control. A bioassay carried out sowing sugar beet seeds revealed a clear protection effect in the pots amended with *B. carinata* meal but also pre-treated with a spore suspension of the selected *Trichoderma* isolate. Treatments by only seed meal or only *Trichoderma* gave lower protection levels. These findings seem to highlight the existence of an additive effect of *Trichoderma* and *B. carinata* meal in reducing sugar beet mortality due to *P. ultimum*.

#### Characterizing defense responses in banana induced by endophytic *Fusarium oxysporum* against the burrowing nematode *Radopholus similis*

Alexander Schouten, Kerstin Schäfer, Richard A. Sikora ..... 155-158

**Abstract:** The burrowing nematode *Radopholus similis* is a devastating pest in banana plantations. Roots can be infested on such a scale that the root system rots away, causing the plant to topple over and subsequently leading to a complete loss of the banana bunch. There are currently no means to control infestation by the nematode other than using nematicides that are notoriously environmentally unfriendly. Recently, it was shown that certain non-pathogenic strains of the endophytic fungus *Fusarium oxysporum* can systemically protect the banana plant against *R. similis* colonization. Although it is assumed that the fungus initiates certain plant defense responses, there is at the moment no data on the exact mechanism. We therefore aim at characterizing the plant defense responses using molecular approaches, when the banana is colonized by the nematode, the endophytic fungus or the combination of the two. The *in planta* accumulation of salicylic acid or jasmonic acid and NPR1 and the PR protein coding transcripts can serve as markers to determine whether induced systemic resistance (ISR) or systemic acquired resistance (SAR) are playing a key role in the defense mechanism. Furthermore, by applying compounds like salicylic acid or jasmonic acid, SAR or ISR in the roots may be mimicked, thus giving clues on the required induced plant response to control *R. similis* infestation. This knowledge serves as a first step in dissecting the involved defense mechanisms and can therefore support the development of strategies to further improve the control of *R. similis* by means of endophytic fungi in banana.

#### Biocontrol of root-knot nematodes by *Trichoderma* – modes of action

Edna Sharon, Ilan Chet, Meira Bar-Eyal, Yitzhak Spiegel ..... 159-163

**Abstract** Isolates of *Trichoderma asperellum* (T 203, T 44, T GH11), *T. atroviride* IMI206040 and *T. harzianum* 248 exhibited biocontrol activity against *Meloidogyne javanica* in soil experiments. Parasitic interactions were demonstrated *in planta*: females and egg masses dissected from tomato roots grown in T203-treated soil were found to be parasitized by the fungus. *Trichoderma* that colonizes the root surface may also interact with penetrating second-stage juveniles (J2s). Conidia attachment and parasitism of fungi on the nematode were examined *in vitro*: egg masses, their derived eggs and second-stage juveniles (J2) were parasitized by *T. asperellum* 203, 44, and *T. atroviride* following conidia attachment. *T. asperellum* GH11 attached to the nematodes but exhibited reduced penetration, whereas growth of *T. harzianum* 248 attached to egg masses was inhibited. Only few conidia of the different fungi were attached to eggs and J2s without gelatinous matrix; the eggs were penetrated and parasitized by few hyphae, while J2s were rarely parasitized by the fungi. Coiling and appressorium-like structures were observed during parasitism. Gelatinous matrix suspension agglutinated *T. asperellum* 203 and *T. atroviride* conidia, a process that was Ca<sup>2+</sup> dependent. Conidia agglutination was reduced by carbohydrates, including fucose, as was conidia attachment to the nematodes. Fucose-specific antibody and lectin enhanced conidia attachment to the nematodes, suggesting the involvement of carbohydrate-lectin based interactions, in the attachment process. All but *T. harzianum* could grow on the gelatinous matrix, which enhanced conidia germination. A biomimetic system based on gelatinous-matrix-coated nylon fibers demonstrated the role of the matrix in parasitism. *T. asperellum* 203, 44 and *T. atroviride* produced metabolites that immobilized J2s in the presence of the matrix. *Trichoderma* proteolytic and chitinolytic activities were also induced during nematode parasitism. Indirect interactions potentially involving systemic induced resistance mechanisms were demonstrated on split root systems with T 203: significant reductions in root

penetration, galling indices, egg production and nematode development inhibition were recorded in the root halves that were not directly treated with *Trichoderma*.

#### Root exudates as important factor in the *Fusarium* – host plant interaction

*Siegrid Steinkellner, Roswitha Mammerler, Horst Vierheilig* ..... 165-168

**Abstract:** *Fusarium oxysporum* strains are known as major pathogens on tomato, an important vegetable crop worldwide. Chemical signals from plants and/or microbes seem to have an important function in determining the positive or negative outcome of interactions in the rhizosphere. However, there is still a lack in the understanding of how root exudates affect the development of fungal propagules in the rhizosphere. We found that tomato root exudates were modified by different *Fusarium oxysporum* strains. The results indicate that the *F. oxysporum* strains pathogenic on tomato Fol 007 and Forl 101587 and the non pathogenic *F. oxysporum* strains Fo 135 and Fo 47 trigger different responses in tomato plants.

#### Operalizing soil quality

*Aad J. Termorshuizen, Peter van Erp, Renske Landeweert, Arjan Reijneveld,*

*Roel Staps, Peter Veenhuizen, Petra C.J. van Vliet, Jos Wubben* ..... 169-172

**Abstract:** The idioms ‘soil quality’ and ‘soil health’ are regularly subject to discussions about their definition and operationalization. Soil quality depends on the goal of soil use. Depending of the goal, certain soil functions are needed. To quantify these soil functions, indicators need to be defined, and computation rules that relate the indicators to soil function. Being an important component of soil quality, the soilborne plant pathogens are discussed in more detail. For the development of advise systems on plant pathogens, the agricultural and horticultural service laboratory Blgg follows three different pathways concurrently: (1) the prediction of damage based on pathogen density and other factors affecting pathogen dynamics, (2) tracking and analyzing past behaviour of pathogens on given fields, and (3) identifying yield problems using satellite biomass maps.

#### Saprotrophic basidiomycete *Hypholoma fasciculare* affects fungal and bacterial community composition in colonized wood and soil.

*Vendula Valášková, Jaroslav Šnajdr, Pauline K. Gunnewiek, Wietse de Boer,*

*Petr Baldrian* ..... 173-176

**Abstract:** *Hypholoma fasciculare* is a common litter-decomposing saprotrophic basidiomycete. It is able to degrade wood as well as different types of leaf litter and to colonize nonsterile soil. *H. fasciculare* caused a substantial reduction of diversity of the fungal community (by approximately 40%) and changes its composition compared to nonsterile noncolonized soil during the colonisation of microcosms with a reconstructed forest soil profile. The uppermost L (litter) layer, where the fungus produced most mycelial mass, was more influenced than the deeper O and Ah layers. In soil with *H. fasciculare* single major bacterial phylotype was present, while the control soil contained many bacterial phylotypes without any dominant species. The analysis of bacterial community associated with the natural samples of wood colonized by *H. fasciculare* in the late phase of degradation revealed only low diversity with <10 main colony types, although bacterial counts (colony forming units) were rather high. PCR/DGGE analysis of selected isolates proved only low diversity among isolates. It is apparent that the white-rot fungus *H. fasciculare* exerts a strong selective pressure on the composition of associated microorganisms both in organic soil layers and in wood.

#### Monitoring of pathogenic and nonpathogenic *Fusarium oxysporum* strains during tomato plant infection

*Shamil Z. Validov, Faina D. Kamilova and Ben J.J. Lugtenberg* ..... 177-183

**Abstract:** Monitoring of pathogenic strains of *Fusarium oxysporum* (*Fox*), which cause wilt and rots on agricultural and ornamental plants, is important for predicting and monitoring the disease outbreaks. Since both pathogenic and non-pathogenic strains of *Fox* are ubiquitous and are able to colonize plants, *Fox* DNA detected in plant material is not the ultimate proof for an ongoing infection which will cause damage to the plant. We followed the colonization of tomato plants by strains *Fox* f. sp. *radicis-lycopersici* ZUM2407 (a tomato foot and root rot pathogen), *Fox* f. sp. *lycopersici* 004 (causing tomato wilt), *Fox* f. sp. *radicis-cucumerinum* V03-2g (a cucumber pathogen) and *Fox* Fo47 (a well known non-pathogenic biocontrol strain). We determined fungal DNA concentrations in tomato plantlets by quantitative PCR (qPCR) with primers complementary to the Intergenic (IGS) Spacer region of these four *Fox* strains. Two weeks after

tomato seedling inoculation with these *Fox* strains the DNA concentration of *Forl* ZUM2407 was five times higher than that of the non-compatible pathogen *Forc* V03-2g and 10 times higher than that of *Fo47*. In three week old plantlets the concentration of *Forl* ZUM2407 DNA was at least ten times higher than those of the other strains. The fungal DNA concentration, as determined by qPCR, appeared to be in good agreement with data of the score of visible symptoms of tomato foot and root rot obtained three weeks after incubation of tomato with *Forl* ZUM2407. Our results show that targeting of the multicopy ribosomal operon gives highly sensitive qPCR reaction for the detection of *Fox* DNA. Since formae speciales of *Fox* cannot be distinguished by comparison of ribosomal operons, detection of *Fox* DNA is not evidence of plant infection by the compatible pathogen. Nevertheless, the observed difference in plant colonization between pathogenic and non-pathogenic strains revealed by our present results strongly suggests that a concentration of *Fox* DNA in plant material above a certain threshold level is due to proliferation of pathogenic *Fox*.

#### Lignin enhances mycoparasitism of *Rhizoctonia solani* and *Botrytis cinerea* sclerotia

*Sarah Van Beneden, Joachim Audenaert, Greet De Backer, Monica Höfte* ..... 185-190

**Abstract:** *Rhizoctonia solani* and *Botrytis cinerea* are two important soil-borne pathogens which cause damage to many crops. They both produce sclerotia, by which they can survive in soil for many years. In this study, the effect of lignin incorporation in soil on the viability of the sclerotia was investigated. When sclerotia of *R. solani* (AG1-1B) were added to soil containing 1% of lignin, their viability was reduced with 60% compared to the control. Remarkably, the sclerotia appeared to be more parasitized by other fungi, mainly *Trichoderma* and *Fusarium*. Similar results were achieved with *Botrytis cinerea* sclerotia. For *Botrytis*, the results were not always consistent; probably the microbial population / activity in the soil plays an important role. When applying lignin in combination with different *Trichoderma* strains, an increase in mycoparasitism of the sclerotia was seen. However, this effect was not observed with all tested strains; it appears to be strain/species-dependent. Although the number of parasitized sclerotia was higher in soils containing lignin, no increase in the total *Trichoderma* population was observed. Possibly, an explanation can be found in the lignin-melanin hypothesis.

#### Comparison of four tillage systems in organic farming. Effect of soil structure modification and organic matter repartition on microbial biomass and soil respiration

*Jean François Vian, Joséphine Peigné, Rémi Chaussod and Jean-Roger*

*Estrade* ..... 191-194

**Abstract:** In agricultural field, the environmental conditions of soil micro-organisms and their ability to degrade soil organic matter and release nitrogen are strongly modified by the type of tillage. We studied the influence of 4 tillage systems in organic farming on the soil microbial biomass and its potential activities of C and N mineralization. In order to connect soil structure resulting from these tillage systems with the soil microbial biomass and to extrapolate microbial measurements at the field scale, we adopted a particular sampling scheme based on the morphological characterisation of the soil structure by description of soil profiles. We sampled in three layers (0-7, 7-15 and 15-30 cm) clods with distinct physical characteristics: porous clods with visible porosity ( $\Gamma$  clods) and compacted clods without visible porosity ( $\Delta$  clods). On each clod we measured distinct physico-chemical (C and N content, C/N, pH and total porosity) and microbiological characteristics (soil microbial biomass SMB, C and Nmin). This sampling method revealed the influence of soil structure and organic matter repartition on SMB and its activity and allowed a more precise assessment of tillage managements on the soil microbial functioning during the transition period from ploughing to no ploughing.

#### Characterization of bacteriophages of *Verticillium*-antagonists originated from the strawberry rhizosphere

*Arite Wolf, Sabine Schulze, Horst Neve* ..... 195-199

**Abstract:** Infection by virulent bacteriophages can control the numbers and biocontrol activity of beneficial bacteria introduced in the plant rhizosphere to suppress plant diseases. The occurrence of phages in the rhizosphere of strawberry was investigated using bacterial antagonists of the soilborne plant pathogenic fungus *Verticillium dahliae* Kleb. as host strains. The phage-sensitive antagonists were characterized by their BOX-PCR fingerprint patterns and showed a high genetic relationship. They were identified as *Pseudomonas*-strains by 16S rDNA sequencing. Up to three

phages by each of the phage-sensitive antagonists were isolated considering different plaque morphologies. The phages belonged to the most common phage morphotypes *Myo*-, *Sipho*- and *Podoviridae* with a size of about 75 to 253 nm. Ikosaedric (67 x 67 nm) and prolate (47 x 100 nm) head morphologies were observed. Prolate phages had a tail length of about 153 nm. Using restriction fragment length polymorphism (RFLP) pattern of the phage DNA (*Bst*EII restriction) about 41 genetically different phages were recorded. The genome size of the phage DNA ranged from 23 to 110 kb. Using the antagonistic strains of *Pseudomonas*, the host range of the phages was broad and variable. Despite the low diversity of the host strains the phages showed a high genetic diversity.

#### Selection and partial characterisation of biofumigants for management of *Verticillium* wilt in strawberries

David Yohalem, David Hall ..... 201-206

**Abstract:** *Verticillium* wilt can be a limiting factor in strawberry production. Until recently, it has been managed by fumigation with the now-banned methyl bromide. Biofumigation using green manures is among those approaches with greatest promise for methyl bromide replacement. During 2006, 10 candidate biofumigants were evaluated in the field for their agronomic properties, *in vitro* for the production of fungitoxic decay products and in microcosms for their ability to reduce populations of the pathogen when mixed into heavily infested soils.

All but one of the candidate materials effected significant reductions in pathogen populations 28 d and 56 d after incorporation, as determined by traditional plating methods. Isothiocyanates were detected after incorporation of all cruciferous materials evaluated (*Brassica juncea*, *Sinapsis alba*, *Raphanus sativus*, *Eruca sativa*), although of different kinds and in different proportions. None of the expected prussic acid was detected from either of the evaluated grasses (*Lolium perenne* and *Sorghum sudanense*). Sulphides and related compounds were detected from *Allium cepa*, while several terpenes (1,8-cineole, camphor, 3-carene, pinenes, borneol) were detected from foliage and stems of *Lavandula angustifolia*.

From these preliminary trials, three candidate materials were selected for further testing in grower fields: *Brassica juncea* (ISCI99), *Sinapsis alba* and BioFence, a seed meal prepared from *B. carinata*. Additional experiments will be performed on *Lavandula* spp. Effects on both edaphic and microbiological properties of biofumigated soils will be estimated. Responses of microfloral functional and species diversities and species richness to biofumigation will be compared and evaluated by several methods.

### Mechanisms involved in inducible and constitutive resistance to pests and diseases

#### Priming as a mechanism behind induced resistance against pathogens, insects and abiotic stress

Jurriaan Ton, Sjoerd van der Ent, Marieke van Hulten, Maria Pozo, Vivian van Oosten, L.C. van Loon, Brigitte Mauch-Mani, Ted C. J. Turlings, Corné M.J. Pieterse ..... 3-13

**Abstract:** Upon treatment with a resistance-inducing agent, plants acquire an enhanced defensive capacity that results in a faster and/or stronger defence reaction at the moment the plant is exposed to biotic or abiotic stress. This phenomenon is commonly known as priming and has been associated with different forms of induced resistance. Priming accelerates and increases the plant's ability to activate the defence that is best adapted to resist the stress situation encountered. Under conditions of disease pressure, primed plants exhibit a higher fitness than non-primed plants or defence-expressing plants. Hence, the benefits of priming outweigh its costs in environments where disease occurs. Although priming has been known to occur in plants for decades, most progress in the understanding of this phenomenon has been made over the past few years. Recent insights in the mechanisms behind systemic acquired resistance (SAR),  $\beta$ -amino-butyric acid-induced resistance (BABA-IR), rhizobacteria-mediated induced systemic resistance (ISR), and volatile organic compound-induced resistance (VOC-IR) against insects have revealed various priming mechanisms that protect against different stresses. Whereas SAR and BABA-IR are associated with priming for salicylate (SA)-dependent defence that acts against biotrophic

pathogens, ISR and VOC-IR seem to function through priming for jasmonate (JA)-dependent defence against pathogens and insects. Expression of BABA-IR and ISR against pathogenic fungi and oomycetes is also associated with an augmented formation of callose-rich papillae. This priming response depends on a largely unknown defence pathway, which involves abscisic acid (ABA) and phosphoinositide (PI) signalling, and is thought to target the cellular secretory pathway. Induction of the primed state may be mediated by an enhanced accumulation of signalling compounds, such as transcription factors (TFs) that remain inactive until the plant is exposed to stress. A Q-PCR-based transcription profiling of ~2.200 TF genes in *Arabidopsis* has revealed consistent changes in the expression of certain TF genes directly upon activation of ISR and BABA-IR. We are currently investigating the contribution of these transcription factors to the various priming responses.

#### Priming in induced disease resistance of plants

*Gerold J. M. Beckers, Uwe Conrath ..... 15-18*

**Abstract:** Infection of plants by necrotizing pathogens or colonization of plant roots with selected rhizobacteria often induces an enhanced resistance to further pathogen attack. The resistance can also be induced upon treatment with certain chemicals or by introducing genetic modifications. In the state of induced resistance (IR) plants are primed for quicker and more effective activation of defense responses upon further pathogen contact. Although the phenomenon has been known for decades, most progress in the understanding of priming has been made over the past few years. Here we summarize the present knowledge of priming in some selected IR phenomena of plants.

#### An extract of *Penicillium chrysogenum* induces resistance in *Arabidopsis* independently of known signalling pathways and elicits early defense-related responses

*Barbara Thürig, Georg Felix, Andres Binder, Thomas Boller, and Lucius Tamm ..... 19-24*

**Abstract:** An aqueous extract of the dry mycelium of *Penicillium chrysogenum* (further called 'Pen'), previously shown to protect various crop plants against several diseases (Thuerig et al. 2006), protected *Arabidopsis thaliana* against *Hyaloperonospora parasitica*, *Botrytis cinerea*, *Alternaria brassicicola* and *Pseudomonas syringae* pv. *tomato* DC3000 without having a direct antimicrobial activity. Various mutants of *A. thaliana* were used to test whether Pen induces resistance on one of the known signalling pathways. Pen was still fully protective against *B. cinerea* in transgenes or mutants impaired in the salicylic acid (*NahG*, *npr1*), jasmonic acid (*coi1-1*), and ethylene (*ein2-1*) signalling pathway. Similarly, Pen-mediated resistance against *H. parasitica* was not affected in the mutants *npr1*, *coi1-1* or *ein2-1*. However, its efficacy was reduced in *NahG* plants.

Pen induced early defense-related responses such as an extracellular alkalinisation in cell cultures and ethylene production in leaf slices of numerous mono- and dicotyledonous plant species, including *A. thaliana*, tomato, tobacco and rice. The Pen-elicitor was sensitive to protease digestion but insensitive to other enzymes, suggesting that the elicitor-active region is a protein or a peptide.

From our data on cell cultures and on *A. thaliana* we conclude that Pen contains an unidentified elicitor, most likely a protein or a glycoprotein, inducing resistance via signal transduction pathways different from classical SA/NPR1- or JA/ethylene-dependent pathways.

#### The identification of *PAD2* as glutamylcysteine synthetase highlights the importance of glutathione in plant disease resistance

*Vincent Parisy, Benoit Poinssot, Lucas Owsianowski, Antony Buchala, Jane Glazebrook, and Felix Mauch ..... 25*

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#### Using Q-RT-PCR to determine a defence-related signal signature of maize

*Danielle Karlen, Marco D'Alessandro, Brigitte Mauch-Mani, Ted Turlings, and Jurriaan Ton ..... 26*

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#### Extracellular factors of early basal resistance induced against bacteria

*Eszter Besenyei, Ágnes Szatmári, Zoltán Bozsó, Erika Szabó, Éva Kondorosi, Katalin F. Medzihradsky, Attila Csorba, and Péter G. Ott* ..... 27-30

**Abstract:** Early basal resistance (EBR) is a response of plants to general bacterial constituents (elicitors), amounting in suppression of the hypersensitive and partial suppression of the susceptible reaction. The quickness of EBR raises the possibility that plants have a non-specific system for inhibition of bacterial activity, for example to prevent deployment of pathogenicity factors. As bacterial pathogens remain outside living plant cells, cell wall proteins and/or structural modifications are likely to be involved in the above EBR effects. Using genomic and proteomic approaches in tobacco, *Medicago truncatula* and pepper, several general elicitor-inducible plant extracellular factors were found to be associated with EBR, including chitinases, peroxidases, proteases as well as structural proteins. Their roles in generating a harmful environment for bacteria may include direct enzymatic damage, nutrient, water and osmotic adjustment and mechanical barrier formation.

#### Cytological aspects of oligosaccharide-induced resistance against *Plasmopara viticola* in grapevine

*S. Trouvelot, M. Allègre, J.-M. Joubert, A. Pugin, X. Daire* ..... 31-36

**Abstract:** Under greenhouse conditions an oligosaccharide (OS) was shown to induce resistance in grapevine (*Vitis vinifera*) against *Plasmopara viticola* (downy mildew). A spray application reduced the severity of the disease by 60-80 %. Following OS application, foliar tissues underwent significant ultrastructural and biochemical modifications, detectable only after the pathogen inoculation. This included H<sub>2</sub>O<sub>2</sub> production at the infection site, the deposition of phenolic-enriched material, the restriction of pathogen development in foliar tissues and the inhibition of its sporulation. Moreover our observations suggested that OS, in grapevine, acted like a priming agent.

#### BABA induced resistance in grapevine

*Ana Slaughter, Mollah Md. Hamiduzzaman, Gabor Jakab, Brigitte Mauch-Mani, and Jean-Marc Neuhaus* ..... 37-41

**Abstract:** Grapevine (*Vitis vinifera*) is a major fruit crop worldwide and is affected by many diseases. Downy mildew, caused by the oomycete *Plasmopara viticola* leads to great damage and yield losses in grapevine if no protective measures are taken. The majority of the traditional cultivars that are planted are susceptible to this disease, necessitating the intensive use of chemicals to limit the damage in vineyards. One possible solution would be the activation of a plant's own defense system, known as induced resistance.  $\beta$ -Aminobutyric acid (BABA) has previously been shown to induce resistance against many oomycetes. It was observed that the protective effect of BABA in *Arabidopsis* was due to the potentiation of natural defense mechanisms against biotic stresses, a phenomenon referred to as priming. Priming is the capacity of a plant to express a faster and stronger basal defense response upon pathogen infection. BABA was used to induce resistance in grapevine. The resistance depended to a large extent on the deposition of callose. A strong reduction of mycelial growth and sporulation in the susceptible cultivar Chasselas was observed. Initial expression profile by microarray of BABA-treated Chasselas will be discussed.

#### Does BABA prime early signalling events in grapevine defence during elicitation and *Plasmopara viticola* infection?

*R. Dubresson, C. Dubreuil, B. Poinssot, J.-M. Neuhaus, A. Pugin, and B. Mauch-Mani* ..... 43-47

**Abstract:** Elicitor treatments or pathogen attack induce early signalling pathways in plants and cell suspensions.  $\beta$ -aminobutyric acid (BABA) has been shown to prime specific defence pathways in plant upon exposure to biotic or abiotic stress. Here we report that BABA priming of grapevine cell suspensions and/or plants treated with elicitors or pathogens did not induce the entire early signalling pathways. BABA primed the production of active oxygen species (AOS) in both cell suspensions and leaves but did not affect nitric oxide (NO) or Ca<sup>2+</sup> levels in cell suspensions.

Studying constitutive and inducible defence of Bt oilseed rape grown under elevated atmospheric CO <sub>2</sub> and ozone concentrations <i>Sari Himanen, Anne-Marja Nerg, Anne Nissinen, Guy M. Poppy, C. Neal Stewart Jr., and Jarmo K. Holopainen</i> .....	49-52
<b>Abstract:</b> Cruciferous plants have distinctive secondary defence against generalist herbivores by glucosinolates and indirect defence, which targets also specialist herbivores. Transgenic Bt plants are the first commercial GM applications conferring specific pest resistance. A transgene coding for Lepidopteran specific <i>CryIAC</i> Bt toxin was inserted into oilseed rape ( <i>Brassica napus</i> ssp. <i>oleifera</i> ) genome, and we are studying if this transformation can result in any changes for the secondary defence of oilseed rape. Concentrations of atmospheric CO <sub>2</sub> and tropospheric ozone, as components of global climate change, are continuously increasing and in the future, this can influence crop productivity and plant-insect interactions. Therefore, we are also investigating if the secondary defence of Bt oilseed rape is changed under elevated CO <sub>2</sub> and O <sub>3</sub> , compared to the parent line.	
Activation of plant defence response contributes to the antiviral activity of Diocin 2 from <i>Phytolacca dioica</i> <i>Franco Faoro, Barbara Conforto, Antimo Di Maro, Augusto Parente, and Marcello Iriti</i> .....	53-57
<b>Abstract:</b> Using the patosystems <i>Nicotiana tabacum</i> , cv. Samsun NN - tobacco mosaic virus (TMV) and <i>Phaseolus vulgaris</i> - tobacco necrosis virus (TNV), we demonstrated that Dioicin 2 (D2), a type-1 ribosome-inactivating protein (RIP), possesses a strong antiviral activity, expressed only <i>in vivo</i> , when both RIP and virus were contemporaneously inoculated in the same leaf (either in the same surface, or separately in the adaxial and abaxial surfaces), but not in the upper leaves. This suggests that D2 cannot translocate into the phloem, nor induce SAR signals. Moreover, D2 alone induced localized H <sub>2</sub> O <sub>2</sub> accumulation in treated tissues but not cell death, which was instead elicited by the concomitant presence of the virus. In that case, some ultrastructural features, such as mitochondrial swelling and nuclear disorganization seem to indicate that programmed cell death phenomena were undergoing. In D2 treated leaves, virus replication still occurred, but was restricted to very few cells and virus lesions remained invisible. Thus, it is likely that D2 antiviral activity is the result of the combined effect of its deadenylation properties on cell and viral nucleic acid and the activation of plant own defense response.	
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## Evolutionary and ecological aspects of plant resistance / deployment strategies for durable resistance within integrated crop management

### Linking aboveground and belowground inducible plant resistance

*T. Martijn Bezemer* ..... 65-71

**Abstract:** Induced resistance of plants against pests and diseases via plant defense responses is well documented and can occur aboveground, in the leaves, and belowground in the roots. A number of recent studies have shown that soil-borne pests can also induce plant resistance aboveground and *vice versa*. Since plants are frequently exposed to aboveground and belowground pests simultaneously, interactions between induced plant defense responses in the foliage and in the roots can have major implications for induced resistance. On the one hand, induction in one plant part may result in systemically induced resistance in the other part. On the other hand, simultaneously occurring aboveground and belowground induced plant defenses may interfere, for example, when the activities of root feeders alter the effectiveness of induced resistance against foliar herbivores. Such aboveground-belowground interactions can also potentially influence tritrophic interactions between plants, herbivores and their natural enemies such as parasitoids or predators.

### Inducible defenses in food webs: models and experimental data

*Irene van der Stap, Matthijs Vos, and Wolf M. Mooij* ..... 72

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### Exploring the barley-Magnaporthe pathosystem

*Nina Zellerhoff, Marcus Jansen, and Ulrich Schaffrath* ..... 73-76

**Abstract:** *Magnaporthe oryzae* is a major pathogen of rice (*Oryza sativa* L.) but is also able to infect other grasses including barley (*Hordeum vulgare* L.). Recently, we discovered that barley genotypes expressing a high degree of resistance against powdery mildew are hypersusceptible against *M. oryzae*. To our knowledge, this was the first example showing that manipulation of the defence potential of a plant which confers resistance against a particular pathogen may favour the development of other diseases. This manuscript is written to give a short survey on research work from our group on the interaction of barley with different species of the *Magnaporthe* species complex, thereby highlighting mechanisms of the basal defence machinery of barley.

### Identification and expression of genes related to herbivory

*Colette Broekgaarden, Roeland E. Voorrips, Marcel Dicke, and Ben Vosman* ..... 77

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### Integrated control of Cucurbit powdery mildew, *Podosphaera xanthii*, (syn. *Sphaerotheca fuliginea*) using resistant cultivars, resistance inducing agents and hyperparasites

*Nikolaos Fanourakis, Anastasia Tampakaki, Maria N. Fanouraki, and Nikolaos*

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### Quantifying cost and benefits of induced systemic resistance in a clonal plant network

*Sara Gómez, Vít Iatzel, Yolanda Verhulst, and Josef Stuefer* ..... 79

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## Chemical ecology / trophic interactions; associations of phenotypes and genotypes

### Communicating plants: Ecological consequences of induced responses to herbivory

**André Kessler** ..... 83-92

**Abstract:** The study of plant-insect interactions is exemplary among the integrative biological research fields and succeeds by unifying the research efforts on the cellular and organismal level with those on the whole plant and community level. This integrative research approach will help to understand the mechanisms of plant-insect interactions and will thus allow the utilization of the plants' natural defenses in agriculture. Here, I highlight studies of herbivore-induced responses of the wild tobacco plant *Nicotiana attenuata* and emphasize both, the role of using chemical and molecular tools in ecological research and the importance of a profound knowledge of the natural history of species when studying plant-insect interactions. In particular I will focus on recent results on the role of herbivore-induced volatile organic compound emission in mediating organism interactions in nature and the potential value of indirect plant defenses for modern agriculture.

### Plant Responses to Caterpillar Footsteps, Chewing and Secretions

**Gary W. Felton and Michelle Peiffer** ..... 93

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### Induction of systemic acquired resistance in *Zea mays* is compatible with plant-parasitoid mutualism

**Michael Rostás, and Ted C. J. Turlings** ..... 94

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### Genotypic manipulation of infochemicals to study chemical ecology and community ecology of herbivore-induced plant volatiles

**Tjeerd A.L. Snoeren, Peter W. de Jong, and Marcel Dicke** ..... 95

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### Volatiles of endophytic bacteria modify defences of maize plants against insects and pathogens

**Marco D'Alessandro, Jurriaan Ton, Jakob Zopfi, and Ted Turlings** ..... 96

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### Factors influencing root colonization by the beneficial strain *Pseudomonas chlororaphis* O6

**Anne Anderson, and Y-C Kim** ..... 97-100

**Abstract:** *Pseudomonas chlororaphis* O6 (PcO6) is an aggressive colonizer of plant roots under laboratory and field conditions. Root colonization is beneficial to the plant, inducing systemic protection against bacterial, fungal and viral pathogens. Antimicrobial phenazines and hydrogen cyanide are produced by PcO6 under GacS-dependent acyl homoserine lactone regulation. Phenazines are produced predominantly in the seed zone of a colonized barley seedling. Mutations in *dctA*, encoding a transporter for carboxylic acids and in *edd*, eliminating catabolism of sugars through the Entner-Doudoroff pathway and reduces colonization early and later in colonization. These findings suggest to us that both organic acids and sugars are used during colonization. Reduced colonization in both *dctA* and *edd* mutants correlated with lessened ability to induce systemic resistance to the soft rot pathogen. Systemic protection against soft rot also is correlated with production of butanediol, a presumed product of fermentation. Butanediol production correlated with increased leaf surface production and induced resistance and is dependent on *gacS* activity. GacS regulates negatively the production of IAA from tryptophan and the shift in phenotype to a highly hydrophobic cell variant that is effective in generating biofilms in minimal medium conditions. These observations illustrate several ways in which the Gac system in *P. chlororaphis* O6 regulates important ecological features in this bacterium.

### Changes in free amino acids induced in cucumber plants by spider mites and plant growth promoting rhizobacteria (PGPR)

**Anna Tomczyk** ..... 101-104

**Abstract:** The experiment was conducted on glasshouse cucumber cv. Corona. Plants were cultivated either in the presence of *Pseudomonas fluorescens* (PGPR) or without these bacteria. Half of the bacterized, as well as non-bacterized plants were infested with *Tetranychus cinnabarinus* Boisd. In all groups of plants, free amino acids were identified and their content was estimated. The total content of free amino acids strongly increased in both spider mite- damaged and PGPR treated plants. Spider mite feeding on bacteria-treated plants caused a lower increase in

free amino acids as compared to untreated plants, however the density of the spider mite population was also lower on bacterized plants as compared to non-bacterized. Amounts of all free protein amino acids strongly increased as a result of spider mite feeding on the leaves of non-bacterized plants as compared to bacterized, while content of non-protein amino acids was decreased in mite-infested as well as in bacteria-treated plants. A high increase of aromatic amino acids was observed in mite-infested plants, however this was less evident when mites fed on bacteria-treated plants. Significant differences in the concentration of proline were found between spider mite-damaged and control as well as bacteria-treated plants.

#### Changes in phenolic acids of cucumber, induced by plant growth promoting rhizobacteria (PGPR) in relation to infestation with spider mites

*Anna Tomczyk* ..... 105-108

**Abstract:** The influence of plant growth promoting rhizobacteria (PGPR), developing in root system of glasshouse cucumber on phenolic acid concentrations in the leaves, important in interaction between host plant and spider mites was studied. Seeds of cucumber plants were inoculated with *Pseudomonas fluorescens*, known as an inducer of plant resistance to some diseases, insects and spider mites. The increase in phenolic acids content was observed in mite and bacteria treated plants as compared to control plants. It was more expressed in young leaves. The plant response was connected with induction of cucumber resistance to spider mites. Changes in the level of phenolic compounds in cucumber leaves, induced by PGPR, can be responsible for the decrease in mite population observed in the previous studies conducted by the author. They can be of some importance in spider mite – cucumber plant interactions.

#### Host spruce quality and its effect on spruce spider mite (*Oligonychus ununguis* Jacobi, Acari: Tetranychidae) biology

*Malgorzata Kielkiewicz, Ewa Puchalska, and Barbara Czajkowska* ..... 109-118

**Abstract:** Comparative studies on chemical composition of needles of Serbian spruce (*Picea omorika*), white spruce (*P. glauca* ‘Conica’) and blue spruce (*P. pungens*) and the effect of host quality on the spruce spider mite (*Oligonychus ununguis*) was carried out. It was stated that fecundity of *O. ununguis* was positively correlated with concentration of reducing sugars, and negatively with concentration of total soluble phenolics in needles of the investigated species of spruce. To the contrary, mortality of the mites was positively correlated with concentration of the total phenols and negatively with concentration of reducing sugars.

The lowest total amount of essential oils was found in needles of *P. omorika*, whereas the one in needles of *P. pungens* and *P. glauca* ‘Conica’ was twice and five times higher, respectively. There were also marked differences in quantities of particular single components of essential oil mixtures among spruces. Needles of *P. glauca* ‘Conica’, being attractive food for *O. ununguis*, distinguished themselves by the highest quantities of camphor, limonene and  $\beta$ -myrcene. Thus, these three monoterpenes may play an important role in spruce spider mite success on white spruce. Needles of *P. omorika*, reluctantly populated and attacked by *O. ununguis*, did not show the presence of camphor and of three other monoterpenes (sabinene,  $\gamma$ -terpinene, *p*-cymene) found in needles of spruces readily accepted by the mite. Moreover, the percent contribution of bornyl acetate, borneol,  $\beta$ -pinene, methyl salicylate, citronellol, geranyl acetate and linalool in the total amount of oil of *P. omorika* was significantly higher than in total amount of oils of the remaining two spruces. Results obtained in these studies indicate that inadequate host spruce quality affects *O. ununguis* bionomy and its abundance reported elsewhere.

## Types of resistance important for plant breeders and possible contribution of inducible resistance

### Breeding for inducible resistance against insects – applied plant breeding aspects

*Inger Åhman* ..... 121-130

**Abstract:** Many of the pre-requisites necessary for breeding plants with inducible resistance to pests are no different from breeding for constitutive resistance. In both cases it is necessary to have resistance genes giving high enough yield gains from pest protection, efficient selection methods and means of introducing resistance genes into agronomically acceptable plant material. In addition, insect resistance traits need to be neutral or positive to non-target organisms. In inducible resistance, there is also the need for proper timing and specificity of induction. Tentatively the ideal inducible resistance is triggered by specific cues which rapidly induce specific resistance traits with long duration relative to the sensitive period of the crop and which give a systemic plant response. It takes knowledge to develop appropriate selection methods for resistance and in the case of inducible resistance we also need knowledge about how the inducing factors are operating. Inducing cues may come from insect activities on the plant (e.g. feeding, oviposition), from neighbouring plants or from manmade chemical formulations of elicitors. Plant selections can be based on plant damage levels, insect numbers, insect responses, plant resistance traits and/or molecular characteristics of the plant genome.

Breeding for insect-inducible resistance to insects has been applied in the form of rapidly induced highly specific resistance in insect–crop combinations where there are gene-for-gene relationships, such as with the Hessian fly and the Russian wheat aphid in wheat. To my knowledge there are no examples of traditional breeding where less specific types of resistance; induced by insects, by neighbouring plants or by chemical formulations; have been deliberately bred into commercial cultivars. It is likely that the accumulating knowledge about the mechanisms of induced resistance will find applications in cultivars produced by genetic engineering.

### Deployment strategies for crops with inducible resistance

*Adrian Newton, Gary Lyon, Graham Begg, Jiasui Zhan, David Guy, Dale Walters*

..... 131-135

**Abstract:** In the field induced resistance in current varieties of crop plants is a normal defence component but it can be further exploited either by a) treating with a resistance elicitor, b) using genotypes which possess a stronger inducible response, or c) selectively encouraging pathotypes that induce it.

Characteristically, resistance elicitors are unreliable as unknown environmental factors sometimes cause substantial loss of efficacy. Furthermore, different crop genotypes, such as cereal varieties, express differential degrees of inducible resistance with given elicitors. As elicitors may act via different receptors it seems advisable to use a combination of elicitors to combine different modes of action to improve efficacy, although trial data to validate this is lacking. However, gene expression data from field-grown plants indicates considerable activation of defence-related pathways so opportunities for further specific pathway induction may be limited.

Growing mixed genotypes of plants would be the norm in many natural ecosystems, but is uncommon in most crops grown as high input monocultures. Nevertheless, there is considerable evidence that cultivar mixtures reduce disease, increase yield, and deliver stability in yield and quality. This is through the spatial effects of dilution of susceptible plants and barriers of resistance plants, and through induced resistance. The latter is achieved through cross-over of spores virulent on one component but avirulent on another, and is thus most effective for pathogens with a 'simple' and specific race structure such as some obligate biotrophs on cereals where its contribution has been estimated. However, mixtures are effective against hemi-biotrophic pathogens with little race-specificity too, and critical to all mixture efficacy is component number, proportions and spatial deployment strategy. For example, a multi-component 'patchy' mixture is likely to be more efficacious than just two or three components in a homogeneous mixture. The scale and degree of patchiness must be a compromise between the parameters required for individual pathogen species control and resource exploitation, the former being determined by pathogen dispersal gradient.

The stability of disease control through exploitation of induced resistance is dependent on its costs, both to the pathogen and to the plant. Modelling demonstrated how the balance can be tipped towards disease control through spatial resistance deployment strategies in mixtures. It can be argued that resistance elicitors will not select for pathotypes that will erode their efficacy as they do not act directly against the pathogen, although experimental evidence indicates this may not always be the case.

- Response of *Solanum stoloniferum* to infestation by the green peach aphid: Induced resistance and gene expression  
*Adriana E. Alvarez, Viviana Broglia, Anahí Maitén Alberti D'Amato, W. Fred Tjallingii, Marcel Dicke, and Ben Vosman* ..... 136  
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- Increased fungal resistance of crops by pathogen induced over-expression of plant resistance genes  
*Klaus Schmidt, Maike Pflugmacher, Waltraud Brieß, Simone Klages, Corinna Rohlf, Anja Mäser, Jeanette Kurrasch, Bernd Holtschulte, Bernd Truberg, Reinhard Nehls, and Dietmar J. Stahl* ..... 137  
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- Derailed stylet mechanics during plant penetration by aphids and its potential as a mechanism of host plant resistance  
*W. Fred Tjallingii, Adriana E. Alvarez, Ben Vosman, and Th. Hogen Esch* ..... 138  
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## **Biotechnology approaches to breeding for (inducible) resistance / tools for biotechnology**

- Critical analysis of Bt-crops as essential element of sustainable insect control  
*Orlando de Ponti* ..... 141  
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- Using pathogen genomics to investigate the induction and manipulation of inducible plant disease resistance  
*Paul R.J. Birch* ..... 142  
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- Induction of defense responses and pathogen resistance in *Arabidopsis* by yeast elicitors  
*Ines C. Raacke, Uta v. Rad, Martin J. Mueller, and Susanne Berger* ..... 143-145  
**Abstract:** Treatment of *Arabidopsis* plants with yeast suspension increases phytoalexin accumulation, PR gene expression and resistance against a subsequent infection with *Pseudomonas syringae* and *Botrytis cinerea*. This protection effect of yeast against *P. syringae* was not detectable in mutants in the salicylic acid pathway indicating that this pathway is important for the protection. In contrast, the increased resistance against *B. cinerea* was present in mutants in the salicylic acid pathway, jasmonic acid pathway or camalexin biosynthesis. This suggests that none of the pathways tested is indispensable for the yeast-mediated protection against this fungus.

- Inducible disease resistance involves an apoplastic cysteine protease cathepsin B  
*Eleanor Gilroy, Edward Venter, Hazel McLellan, Ingo Hein, Katarina Hrubikova, Maria Holeva, Petra Boevink, Gary Loake, Christophe Lacomme, and Paul Birch*..... 146  
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- Proteomic analyses to understand pathways of resistance to aflatoxin accumulation in developing maize ears  
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- Activation of defense responses in *Arabidopsis* by non enzymatically formed oxylipins  
*Christoph Grun, Martin J. Müller, Susanne Berger* ..... 148  
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Impact of transgenic cucumbers expressing the thaumatin II gene on the occurrence of arthropod fauna

Malgorzata Kielkiewicz, Janina Gajc-Wolska, Maria Szwacka, and Stefan Malepszy ..... 149-153

**Abstract:** The present study report on the impact of genetically modified cucumbers expressing the thaumatin II gene on the occurrence of arthropod fauna. Four lines of transgenic cucumbers (T224, T225, T212, T210) derived from the inbred line of *Cucumis sativus* L. cv. Borszczagowski (line B) and exhibiting relatively high/low level of thaumatin II protein in the leaf were weekly evaluated under field conditions on insect and mite pests, and their natural enemies abundance. Fewer onion thrips (*Thrips tabaci*), cotton aphids (*Aphis gossypii*) and two-spotted spider mites (*Tetranychus urticae*) settled and developed on transgenic lines than on the control, line B. More leaves damaged by tarnished plant bug (*Lygus rugulipennis*) were recorded on non-transgenic than transgenic plants. The coexistence of herbivores and their natural enemies as *Coccinella septempunctata*, *Aphidoletes* sp., *Chrysoperla* sp., *Aeolothrips intermedius* and *Orius minutus* on studied cucumber lines was observed. Our results indicate that transgenic cucumber plants expressing the thaumatin II gene affect the abundance of piercing-sucking pests. However, there was no clear relationship between herbivores density and leaf thaumatin level.

Transgenic plantlets of grapevine (*Vitis* spec.) provide a look insight of host-pathogen interaction

T. Seibicke, and H.-H. Kassemeyer ..... 154

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