

## Impact of grapevine downy and powdery mildew diversity on efficacy of phosphonate derivatives (fosetyl-AL and fertilizer PK2) and salicylic acid analog (BTH) described as stimulators of plant defences

Marie-Cecile M.-C. Dufour, Marie-France Corio-Costet

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### **IOBC-WPRS Bulletin Vol. 83, 2012**

yield of strawberry plants.

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attacker has passed constitutive barriers, several plant hormones are produced leading to a local and systemic defense response that will counteract the invader. It is known that the hormone jasmonic acid (JA) plays a major role in the defence against insects and necrotrophic pathogens. JA can activate different sets of JA-responsive genes, depending on other (attacker specific) signaling molecules that are simultaneously produced upon attack (e.g. the phytohormones abscisic acid (ABA) and ethylene (ET)). Transcription factors (TFs) play an important role in the regulation of the differential JA responses. In *Arabidopsis thaliana* the TF MYC2 was identified as key regulator of wounding specific JA-regulated responses. *MYC2* gene expression is activated by both JA and ABA and repressed by ET. In contrast, several ERF-type TFs such as AtERF14, ERF1 and ORA59 are activated by JA and ET and repressed by ABA. The MYC2 TF is believed to be important in defense against insects. Upon caterpillar feeding, a plant mutated in MYC2 shows a shift in its JA-dependent transcriptional profile compared to the wild type. In wild-type Arabidopsis, the MYC2-dependent *VSP2*-branch of the JA response is activated. Although this shift in transcription pattern appeared to have no direct influence on the growth of larvae of the small cabbage white (*Pieris rapae*), caterpillar choice tests revealed that *P. rapae* larvae have a preference for *myc2* mutant plants over wild-type plants. This indicates that activation of MYC2dependent JA responses plays a role in deterring insect herbivores such as *P. rapae*, resulting in less damage to the plant.

#### Resistance induction and priming by plant volatiles

#### Antimicrobial effects of extrafloral nectar (EFN)

# The role of induced defences in the success of an exotic pine: the importance of recognizing your enemies

Abstract: The Enemy Release Hypothesis (ERH) is one of the mostly cited theories to explain how exotic species become invasive out of its natural range. This hypothesis posits that plants species become invasive because of reduced regulation by herbivores in their introduced range. This hypothesis has been widely tested in different plant-animal systems and results are controversial. We investigate whether differences in inducibility between a native (Pinus pinaster) and an exotic pine (P. radiata) may explain the differences in the attack patterns of a local generalist insect herbivore, Hylobius abietis (Coleoptera, Curculionidae). We evaluated the damage rate of this insect in i) in vitro cafeteria experiments, ii) in vivo bioassays, and iii) in two naturally infected genetic trials of both species, jointly planted on a coniferous clear-felled area. Contrary to the ERH predictions, debarked area caused by the pine weevil was significantly greater in the exotic pine in both field trials. However, in vitro bioassays with the same material showed the opposite, and the pine weevil clearly preferred the species with which it has coevolved. No significant differences were observed in the *in vivo* bioassays after a 72h feeding period. The higher resistance of P. pinaster in field conditions could derive from induced resistance mechanisms preferentially elicited in the native species following the insect damage. Indeed, the induction of resin in the stems (the main resistant trait in conifers) after a 72h feeding period was twice in the native than in the exotic pine. These results suggest that the native pine, although constitutively more susceptible, is able to recognize the potential enemy, and elicit the appropriate defence mechanisms, resulting in significantly better defended seedlings. Considering the capability to elicit induced resistance traits against alien and local insects appeared to be essential to correctly interpret the predictions of the ERH.

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#### Factors influencing the inhibition of aphids by $\beta$ -aminobutyric acid

#### 

Abstract:  $\beta$ -aminobutyric acid (BABA) is a non-protein amino acid that confers wide-ranging protection in a number of plant families against a variety of plant pathogens, nematodes and insect herbivores. This paper aimed to clarify some of the conditions under which application of BABA to a host plant inhibits the performance of aphids. With whole plants, application of BABA by spraying or dipping foliage had no effect on aphid performance, whereas application as a root drench caused a reduction in growth of a number aphid species developing on a range of host plants. This suppression was not observed when  $\gamma$ -aminobutyric acid was applied, suggesting the effect is isomer specific. Aphid growth was also reduced on detached leaves where the cut end of the petiole was immersed in BABA-solution, indicating that the plant did not need to be intact for BABA-induced inhibition to occur. Adult aphids maintained on BABA-treated plants exhibited reduced nymph production after 4-5 days. The findings indicate that BABA-induced inhibition of aphids is a very general effect, occurring in all plant species so far tested, against all aphid species, genotypes and life stages.

## The effects of β-aminobutyric acid on seed germination, growth and chemical composition of crop plants

Abstract: DL-\beta-aminobutyric acid (BABA) is a non-protein amino acid that confers wideranging protection in a number of plant families against a variety of plant pathogens and insect herbivores. However, BABA can sometimes produce phytotoxic effects or lead to reductions in plant growth or yield. The performance of insect herbivores maintained on BABA-treated host plants could be affected by stress-induced changes in the plant as well as by enhanced plant defences, so it is important to clarify the effects of BABA on plant functioning. Soaking seeds (Vicia faba, Medicago truncatula, Brassica oleracea, B. rapa and Hordeum vulgare) in BABA solution for 24h had no effect on their subsequent germination. However, application of BABA as a root drench caused reductions in shoot length and shoot fresh weight of *Pisum sativum*, Medicago truncatula, Beta vulgaris, Hordeum vulgare, Solanum lycopersicum and Brassica oleracea. BABA consistently induced an increase in shoot percent dry weight, suggesting an effect on plant water balance. BABA caused numerous changes in the chemical composition of shoot material of Vicia faba and Medicago truncatula, including an increase in the concentrations of H and N. There was also a decrease in K in both plants, which might be another factor associated with plant water balance. The results suggest that, although BABA is known to act via the priming or activation of various defence pathways, there are also a number of physical and chemical changes induced in the host plant that could potentially impact on the performance of insect herbivores.

#### Mycorrhizal symbiosis as a strategy for root parasitic weed control

Juan Antonio López-Ráez, Sabine Christina Jung, Iván Fernandez,

plants colonized by the AM fungus *Glomus mosseae* induce less germination of *Orobanche ramosa* seeds than non-mycorrhizal plants. The results indicate that AM fungi may be used as a suitable tool for controlling root parasitic weeds by reducing strigolactone production by the host plant.

#### Session II. Chances and limits in model systems Oral presentations

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range of microbial pathogens and insect herbivores with different lifestyles and infection or feeding strategies. The evolutionary arms race between plants and their attackers provided plants with a sophisticated defense system that, like the animal innate immune system, recognizes the attacker and responds by activating specific defenses that are specifically directed against the

invader. Recent advances in plant immunity research provided exciting new insights into the underlying defense signaling network. Diverse small-molecule hormones play pivotal roles in the regulation of this network. Their signaling pathways cross-communicate in an antagonistic or synergistic manner, providing the plant with a powerful capacity to finely tailor its immune response to the attacker encountered. Pathogens and insects, on the other hand, can manipulate the plant's defense signaling network for their own benefit by affecting phytohormone homeostasis to antagonize the host immune response.

Plant innate immunity: at the cell wall and beyond

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Role of the *Thctf1* transcription factor of *Trichoderma harzianum* 

in 6-pentyl-2*H*-pyran-2-one production and antifungal activity *M. Belén Rubio, M. Rosa Hermosa, Michelina Ruocco, Matteo Lorito* 

Abstract: The Trichoderma harzianum Thctf1 gene, which shows high sequence identity with a transcription factor gene of Fusarium solani f. sp. pisi, was cloned and characterized. In T. harzianum T34, disruption of the Thctf1 gene by homologous recombination gave rise to transformants that did not show the yellow pigmentation observed in the wild-type strain in plate experiments. In several Trichoderma spp. a yellow pigmentation and a coconut aroma have been related to the production of 6-pentyl-2H-pyran-2-one (6PP) compounds. Prompted by this, we explored whether the loss of pigmentation in the *Thctf1* null mutants of *T. harzianum* could be related to the synthesis of 6PP. Chromatographic and spectroscopic analyses revealed that the disruptants did not produce two secondary metabolites, derived from 6PP and not previously described in Trichoderma genus, that are present in wild-type culture filtrates. Since 6PP is a recognized antifungal compound, this ability was analyzed *in vitro* in both the disruptants and in the wild-type. It was observed that the Thctfl null mutants of T. harzianum had reduced antimicrobial capacity. In vivo assays are also being carried out in order to analyze the tomato plant behaviours in interaction with T. harzianum T34 and the deletion mutants, in the absence or presence of a pathogen. Tomato microarrays are being used to explore the changes in the plant transcriptome during these interactions.

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Impact of environmental factors on PAMP-induced callose

in hydroponically grown Arabidopsis

Estrella Luna, Brigitte Mauch-Mani, Victor Flors, Pilar García-Agustín,

growth medium suppressed basal and PAMP-induced callose deposition, suggesting that reactive oxygen species (ROS) enhance PAMP-induced callose deposition. Since the plant hormone ABA is involved in priming of pathogen-induced callose (Gomez-Gomez & Boller, 2000), sugar signalling (Iriti & Faoro, 2008) and ROS production (Kohler *et al.*, 2002), we investigated the effects of this hormone in our model system. Addition of ABA to the growth medium augmented both basal and PAMP-induced callose. This outcome suggests a positive influence of ABA on callose deposition, consistent with a stimulatory role of ROS in callose deposition. However, this result is not consistent with findings by other labs, who reported suppressive effects by ABA on induced callose deposition (Nishimura *et al.*, 2003). Based on our finding that various environmental factors can influence callose deposition, we propose that the variable effects by ABA are caused by interactions between ABA signalling and other abiotic stress pathways.

OCP3, a new regulator of the Induced Systemic Resistance

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# Session III. Chances and limits in crop protection Oral presentations

Aspects of induced resistance against grapevine downy mildew *X. Daire, S. Trouvelot, B. Poinssot, M. Adrian, M.-C. Heloir and A. Pugin* ...... 119 Abstract only

**Abstract:** Some beneficial microorganisms can reduce disease symptoms through activation of the induced systemic resistance (ISR). Scarce knowledge is available on the efficacy, persistence and fitness cost of ISR in non-model systems. Our aim was to characterize the resistance against *Plasmopara viticola* activated in grapevine by the biocontrol agent *Trichoderma harzianum* T39. T39 activated a systemic resistance and reduced downy mildew symptoms at a level comparable to treatments with benzothiadiazole (BTH). However, if only the treated leaves were considered, T39 induced a lower protection level and a shorter persistence of the effect compared to BTH. In addition, BTH treatments entailed energy costs, which strongly reduced grapevine growth, but T39 treatments did not affect photosynthesis and plant growth. These results suggest the activation of different defense pathways in grapevine after BTH and T39 treatment.

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Differential responses of herbivorous insect species to protease inhibitors from barley and strawberry

Laura Carrillo, Manuel Martinez, Inés Cambra, Marina Gambardella,

Guy Smagghe, Felix Ortego and Isabel Diaz ...... 129-132 Abstract: In this study has been analysed the *in vitro* inhibitory activity of cystatins from barley (HvCPI1 to HvCPI-13) and strawberry (FaCPI-1) against different species of insects that rely on cysteine-like proteases for digestion. These proteins presented different capability to inhibit the activity of cathepsin L-like protease activities in the different species tested, being HvCPI-6 the most effective inhibitor. Gelatin-containing gels showed most of the multiple protease forms detected in the insect extracts were partially or even totally inhibited by this barley cystatin. Besides, the *in vivo* effect of this HvCPI-6 was analysed on two aphid species by feeding assays with supplemented artificial diets.

#### Priming plants for stress resistance: from the lab and the field

### Gerold J. M. Beckers, Michal Jaskiewicz, Yidong Liu, William R. Underwood,

Sheng Yang He, Shugun Zhang and Uwe Conrath ...... 133-137 Abstract: Upon infection by a pathogen or upon treatment with certain commercial fungicides plants can develop resistance to a broad spectrum of pathogens and/or abiotic stress. The induced resistance is frequently associated with the so-called "priming" of cells. Priming is the phenomenon that enables cells to respond to much lower levels of a stimulus in a more rapid and robust manner than non-primed cells (Prime-A-Plant Group, 2006). It has been hypothesized that priming involves accumulation of dormant signaling components that are not used until challenge exposure to pathogens or abiotic stress. However, until recently the identity of such signaling components has remained elusive. We showed that during development of induced resistance in Arabidopsis thaliana, priming is associated with accumulation of mRNA and inactive protein of mitogen-activated protein kinases (MPK) 3 and MPK6. Upon challenge exposure of the plants to Pseudomonas syringae pv. maculicola or infiltration of water into leaves, these two enzymes were more strongly activated in primed plants than in non-primed plants. This elevated activation was linked to enhanced defense gene expression and development of induced resistance. In addition, priming of defense gene expression and induced resistance were lost or reduced in mpk3 or mpk6 mutants (Beckers et al., 2009). Our findings argue that pre-stress deposition of the

signaling components MPK3 and MPK6 is a critical step in priming plants for full induction of defense responses during induced resistance. The role of MPK3 and MPK6 in induced resistance and the potential of priming for modern pest management in the field is illustrated.

Arbuscular mycorrhiza induce systemic resistance against gray mold (Botrytis cinerea) in tomato through priming of JA-dependent defense responses Sabine C. Jung, Javier García-Andrade, Adriaan Verhage, Iván M. Fernández, Juan M. García, Concepción Azcón-Aguilar and María J. Pozo ...... 139-144 Abstract: Arbuscular mycorrhizal fungi (AMF) are soil fungi that form mutualistic symbioses with the roots of about 80% of all terrestrial plants. The association improves plant fitness in terms of nutrition and resistance to biotic and abiotic stresses. We analyzed the susceptibility of mycorrhizal and non-mycorrhizal tomato plants to the necrotrophic fungus Botrytis cinerea, causal agent of gray mold in tomato leaves. Disease severity was lower in mycorrhizal plants. Moreover, the amount of pathogen in the tissues was significantly reduced in those plants.

Similar results were obtained in whole plant and detached-leaf assays, confirming Mycorrhiza Induced Resistance (MIR) to *B. cinerea*. We have analyzed the possible role of jasmonate regulated defense responses in the enhanced resistance. The induction of jasmonic acid (JA)-dependent marker genes in response to *Botrytis* inoculation was higher in mycorrhizal plants. Furthermore, mycorrhizal plants displayed a potentiated response to exogenous application of JA. Altogether, our results suggest that systemic resistance to *B. cinerea* in mycorrhizal plants is associated to priming of JA-dependent responses.

#### Priming in melon plant induced by FEN560, a novel plant extract elicitor

#### in challenge against biotic attackers

Djamel Edine Kati, Sabine Schorr-Galindo, Ziya Gunata,

Abstract: This study describes the priming in induced resistance in melon plants treated with FEN560, a plant extract elicitor derived from Trigonella foenum-graecum that was developed for bio-phytosanitary uses. In order to assess the priming occurrence, two cultivars of Cucumis melo L. (susceptible and resistant cultivars) were artificially infected with Fusarium oxysporum Schlecht. f. sp. melonis (FOM), 24h after FEN560-treatment. To evaluate the systemicity of induced resistance triggered by FEN560, treatments were performed either by spraying the FEN560-solution or through irrigation. As marker of induced resistance, the changes in lipoxygenase (LOX) and peroxidase (POD) activities were monitored. Besides, a comparative study of peroxidase isoenzymes in roots and cotyledons was also performed by iso-electrofocusing (IEF) technique. Results of lipoxygenase and peroxidase kinetic activities and their isoforms showed that FEN560 markedly induced the resistance in both melon cultivars. Activation of the enzymatic markers also depended on the mode of treatment. Interestingly, the induced resistance triggered by the foliar treatment was similar to Systemic Acquired Resistance while the induced resistance triggered by the treatment of roots was comparable to Induced Systemic Resistance. Nevertheless, the priming was localised on the inoculation site (biotic elicitation), mainly observed in susceptible cultivars.

## Implications of nitrogen metabolism in plant basal resistance and priming Gemma Camañes, Victor Flors, Victoria Pastor, Begonya Vicedo,

Javier García-Andrade, Miguel Cerezo and Pilar García-Agustín ...... 151-156 Abstract: Nitrate transporters are membrane proteins in charge of nitrate transport through the roots. Among the nitrate transporters, AtNRT2.1 and AtNRT2.2 are the main transporters involved in uptake of nitrate when it is present at low concentrations through a high-affinity transport system (HATS). Apart from nitrate transport, it is speculated that these transporters, the gene or the protein, may have additional roles in signaling and definition of root architecture. A mutation in both high affinity nitrate transporters (AtNRT2.1 and AtNRT2.2) confers enhanced resistance against Pseudomonas syringae by priming SA dependent responses. Enhancement of SA marker genes PR-1 and PR-5 is correlated with increases of the hormone SA during the first 48h after infection. Interestingly, AtNRT2 induction after three days of nitrate starvation results in enhanced susceptibility in wild-type plants against Pst. In addition, mutant plants showed altered ABA control by Pst. ABA level does not change in atnrt2 during the infection while it is increased in wild type plants. Coronatine less Pst DC3118 produces reduced symptoms on Ws background while it grows as *Pst* DC3000 in *atnrt2.1*. Therefore it seems that *atnrt2* displays some interference with ABA signaling, which probably results in a deficient ABA control by the pathogen. All these results suggest that both transporters may act as environmental sensors of abiotic/biotic stress modifying plant responses to pathogens.

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Possibilities on the use of resistance inducers in tobacco leaf production,	
results of field works in Brazil	
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**Abstract:** The effectiveness of chitosans (CHTs), deacetylated chitin derivatives, as plant resistance inducers depends on a number of variables, such as the molecular weight (MW) and the deacetylation degree (DD) of the oligomers/polymers, the solvent, the pathosystem and the mode of administration. All these aspects are briefly examined and discussed with the aim of giving useful advices for succesful application of CHTs, particularly to control virus diseases.

Mycorrhizal symbiosis as a strategy for root parasitic weed control

Juan Antonio López-Ráez, Sabine Christina Jung, Iván Fernandez,

Juan Manuel García, Harro Bouwmeester and María José Pozo ...... 165-169 Abstract: Parasitic weeds of the genera Striga and Orobanche spp. cause severe damage to important agricultural crops worldwide. Although some promising control methods against these parasitic plants have been developed, new strategies for integrated approaches are still relevant. The lifecycle of root parasitic weeds is intimately associated with their host and is a suitable target to develop such new control strategies. Of particular interest are approaches directed at early stages of the host-parasitic interaction. Strigolactones were first described as germination stimulants for the seeds of these root parasitic plants. In addition, they also act as host detection signals for arbuscular mycorrhizal (AM) fungi. It is well known that AM fungi have a positive effect on plant fitness and on the induction of plant defence responses, conferring resistance to biotic and abiotic stresses. In relation to parasitic plants, it has been recently shown that AM fungal inoculation of maize and sorghum lead to a reduction in Striga hermonthica infection. Moreover, we previously showed that a tomato mutant with a reduced production of strigolactones was less susceptible to Orobanche aegythiaca infection. Here we show that tomato plants colonized by the AM fungus Glomus mosseae induce less germination of Orobanche ramosa seeds than non-mycorrhizal plants. The results indicate that AM fungi may be used as a suitable tool for controlling root parasitic weeds by reducing strigolactone production by the host plant.

Induced resistance in pepper by Fo47 is associated to changes

#### in gene expression

Javier Veloso and Jose Díaz ..... 171-174

**Abstract:** Pepper is an important crop that is used in many ways for gastronomic purposes. There are many diseases that affect pepper plants, being *Verticillium* wilt one of the most important in the region of Galicia (Northwest of Spain). So far, there is not an effective protection scheme based on fungicides or gene-for-gene resistance for this disease, so it is important to explore alternative strategies of control as induced resistance.

In this study we tested the ability of the non pathogenic strain Fo47 to protect pepper plants against *Verticillium dahliae*. This non pathogenic strain of *Fusarium oxysporum* was successfully tested in another plant species against *Fusarium* wilt (Alabouvette & Couteaudier, 1992). In our bioassay we treated pepper plants with Fo47 and then we challenged them by submerging the root in a suspension of *Verticillium* conidia. Symptom evaluation was done by determining the stem length, the fresh and dry weight and the percentage of wilted leaves. A significant protection against *Verticillium* wilt was achieved by inducing the plants with Fo47. Systemic response against a necrotrophic fungus (*Botrytis cinerea*) was also tested, but Fo47 failed to control this pathogen.

An *in vitro* pairing assay was performed to observe the possible interactions between Fo47 and *Verticillium dahlae*. Fo47 inhibited partially the growth of the pathogen.

In order to check if gene expression associated to plant defence is induced by Fo47 in pepper, we took samples of the roots and stem of the plants after Fo47 treatment and after the challenge with *Verticillium*. Gene expression was determined by using quantitative (Real Time) RT-PCR. The genes tested were *CASC1*, a sesquiterpene cyclase related with the synthesis of a phytoalexin, *CACHI2*, a gene that encodes a quitinase and *CABPR1*, a PR-1 protein. Overall, the expression of the three genes was enhanced by Fo47 both before and after challenge. So far, our results suggest that Fo47 control *Verticillium* both by antagonism and induced resistance.

#### Characterization and cloning of pathogen-inducible genes and promoters

of Carica papaya to improve resistance to Phytophthora palmivora

As a first step toward engineering resistance of C. papaya to P. palmivora, defense-related genes and inducible promoters in Carica papaya in response to P. palmivora were characterized in this study. A survey of the root transcriptome and the expression of genes isolated from the roots of C. papaya (cultivar 'SunUp') seedlings were evaluated for regulation by P. palmivora after infection with this pathogen. Twenty-three genes exhibiting predominant root expression were isolated from a cDNA library created from infected root tissues. Sequence analysis revealed a number of genes associated with stress, pathogen and defense-related response. An open reading frame (ORF) encoding a predicted ascorbate peroxidase was found to be up-regulated in leaves, but not in roots. Another peroxidase ORF was down-regulated in roots, while genes predicted to encode a  $\beta$ -1,3-glucanase and ferulate 5-hydroxylase (F5H) were up-regulated in roots. An ORF encoding a hypersensitive-induced response protein was induced by *P. palmivora* in both roots and leaves. Finally, an ORF predicted to encode an aquaporin with normally high root expression was down-regulated following inoculation. Although many host genes regulated during *Phytophthora* infection are associated with the host defense, others are required for pathogenicity. These genes have significance roles in the plant-pathogen interactions, and several early and strong pathogen-inducible promoters for papaya roots will be useful for engineering novel pathogen resistance. Collectively, expression patterns revealed in this study and similar studies can be used to identify host genes regulated by Phytophthora for pathogenicity and host defenses with their associated pathways to provide fundamental knowledge on the mechanisms by which papaya metabolically responds to this pathogen.

#### Imaging techniques for evaluation of the pathogen impact on the host plant

#### Mónica Pineda and Matilde Barón ..... 181-184

**Abstract:** Imaging techniques are currently used for monitoring the plant metabolism and health status. Among them, the most widespread is chlorophyll fluorescence imaging, which can examine photosynthetic performance and evaluate the non-photochemical dissipation processes of the energy non-used in photosynthesis. Multicolour fluorescence imaging can assess both the primary and the secondary metabolism of plants, giving information about the production of several stress-induced phenolic compounds. Thermal infrared cameras provide images of the leaf surface temperature, thus showing transpiration patterns. The set of images obtained combining different imaging techniques could provide "stress signatures", characteristic of every plant-stress factor.

#### **Poster presentations**

Plant growth promoting Rhizobacteria trigger isoflavone metabolism

in early stages of development in *Glycine max* var Osumi

Elena Algar, Ana Garcia-Villaraco, Jorge García Cristobal,

The aim of this work was to evaluate the effects of nine PGPR isolated from different backgrounds to alter IF levels in *Glycine max* var. Osumi. Different experiments were carried out inoculating each strain on two-day old pregerminated seeds sown on sterile pots filled with vermiculite. Six days after inoculation, photosynthesis was measured and seedlings were harvested. Weight of shoots, cotyledons and roots were registered and isoflavones in each organ were analyzed by HPLC.

Although only one strain (N21.4) increased total IF contents as compared to controls, five different behaviours were detected when the daidzein and genistein families were analyzed. N21.4 has shown its ability to trigger defensive metabolism against leaf pathogens to a different extent in the model plant *A. thaliana* and in tomato, and it was a systemic induction in both cases. Interestingly, only one strain caused significant decreases in total IF (M84), and three strains increased IF levels in leaves, two of them coupled to a decrease in roots (N11.37, L81) and one was not accompanied by this decrease (Aur6). All strains triggered IF metabolism so further studies have to be developed since the different beneficial effects of IF through the diet may be due to the different IF profiles and also, they will have different physiological effects on plant performance upon pathogen challenge or for symbiosis establishment.

In conclusion, these are encouraging results from three points of view i) N21.4 increases isoflavones in seedlings; ii) other strains trigger IF metabolism differentially, hence, both facts could be used to prepare food supplements or as enriched standardized foods after full development of the biotechnological procedure and iii) Further studies need to be carried out to relate changes in IF with protection against leaf pathogens, unravelling the underlying mechanisms of the systemic induction.

### Chemical induction of SAR in pea (*Pisum sativum* L.) against pea rust enhances antifungal activity and accumulation of phenolic compounds

peroxidase. However differences in the PR proteins induced were observed between BTH and BABA treatments.

#### Antimicrobial synthetic cationic lipopetides and plant induced resistance

## Elicitors of *Leptosphaeria maculans* inducing resistence to blackleg in oil seed rape Lenka Burketová, Vladimír Šašek, Lucie Lorková, Phuong Dinh Kim

Preventive and post-infection control of *Botrytis cinerea* in tomato plants and fruits by hexanoic acid *Maria de la O Leyva, Begoña Vicedo, Ivan Finiti, Pilar Garcia-Agustin*and Carmen González-Bosch
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**Abstract:** Grapevine is subjected to numerous forms of pathogen aggression, especially downy and powdery mildews (*Erysiphe necator* and *Plasmopara viticola*). To develop new integrated pest management strategies and to understand the impact of alternative methods like plant defence stimulators on the evolution of bioaggressor populations, it is important to investigate the role of genetic variability and the evolution potential of pathogen populations subject to alternative method selection, in order to decrease the risk of resistant populations. The efficacy of two phosphonate derivatives (Fosetyl-Al and PK2, a foliar fertilizer) and benzothiadiazol (BTH), a salicylic acid analog, were assessed on the induction of grapevine defences against various phenotypes of grape downy mildew and various genotype groups of powdery mildews in bioassay: firstly by measuring the growth inhibition of pathogens after treatments with the different products; secondly by monitoring gene expressions coding for enzymes of phenylpropanoids (PAL, STS, CHS, CHI, LDOX, BAN), phytohormones (LOX, ACC, PAL) biosynthesis pathways and genes coding for PR proteins (CHIT4c, PGIP, PIN, GLU, PR1, PR10); and finally, by quantifying secondary metabolite production. Thus correlations between gene expression levels, efficacy and phytoalexin production were obtained.

# Biological protection conferred by *Glomus* spp. and *Bacillus megaterium* against *Meloidogyne incognita* in tomato and pepper

#### 

**Abstract:** The aim of this study was to evaluate the individual capability of 5 PGPR strains, which co-inoculated demonstrated ability to protect rice plants against salt stress, to stimulate the secondary metabolism of plant and protect them against salt stress. The strains used were: *Arthrobacter oxidans* BB1, *Chryseobacterium balustinum* AUR9, *Bacillus* sp. L81, *Aeromonas* sp AMG272 y *Herbaspirillum* sp. DSM6446. Seedlings growing for 7 days in hydroponic culture were inoculated with PGPR, and 7 days after, NaCl was added to the medium to reach a concentration of 3.5g/l. Two days later, the rate of wilt, the peroxidase activity (enzyme linked to stress situations), biometric parameters, and parameters related to plant photosynthetic efficiency (Fv / Fm, NPQ and  $\Phi$ PSII) were measured.

Strains AMG272, L81 and *Herbaspirillum* were able to reduce the rates of withering up to 80%, while BB1 and Aur 9 failed to protect the plants. The strains that protected against the salt stress, altered peroxidase activity and photosynthetic parameters following the guidelines set in the literature of the process called "priming".

## Systemic induction of bean isoflavones by a plant growth promoting Rhizobacteria

consortium against the leaf pathogen *Xanthomonas campestris* pv. *phaseoli A. García-Villaraco, E. Algar, J. A. Lucas García, B. Ramos Solano* 

and F. J. Gutierrez Mañero ...... 225-230

**Abstract:** Plant growth promoting rhizobacteria are non-pathogenic bacteria able to trigger plant's defensive metabolism. In some plant species as legumes, isoflavones (IF) are secondary metabolites relevant for human health and also play a role in plant defense. Although among legumes, only soybeans are known for the high IF contents, beans (*Phaseolus vulgaris*) may represent a considerable input of IF in the diets since they are by far, a lot more popular in the Mediterranean area than soybean. Increasing bean productivity according to environmentally friendly agricultural practices is a challenge that increases its attractiveness if the product has an added value such as a high IF content. This may be achieved with biofertilizers which aim to improve plant nutrition at the same time that the plant's defensive metabolism is elicited. Since PGPR may use different mechanisms to achieve these goals, our rationale was to evaluate if a combination of PGPR with different mechanisms would result in better effects than upon simple applications, evaluating changes in growth and IF contents in early stages of bean development and protection against leaf pathogen.

Two different experiments were carried out to address these goals. A short experiment in which the consortium and the individual PGPR were inoculated on two-day old pre-germinated seeds sown on sterile pots filled with vermiculite. Six days after inoculation, photosynthesis was measured and seedlings were harvested. Weight and height of shoots, cotyledons and roots were registered and IF in shoots (free of cotyledons) and roots were analyzed by HPLC. On the second experiment, pre-germinated seeds were transferred to 500ml pots and inoculated twice with the consortium or the individual bacteria, one upon and the second 12 days after; six days after transplant the second inoculation, plants were pathogen challenged and one week after, disease symptoms were recorded. All strains were able to prime the plant since all decreased plant height in the shirt experiment, indicating that plants detour C metabolism to defensive metabolism compromising growth (Conrath et al., 2002). Despite these changes in total IF were non significant under any treatment, including the consortium, this was not correlated to protection achieved on long experiments. Individual strains performed a lot better than the consortium, that even increased the disease symptoms; interestingly BB1 significantly increased daidzin levels coupled to a decrease on its aglycon and these plants showed the lowest disease incidence (85% protection). Therefore, based on these data, it may be concluded that the consortium does not seem to provide any advantage to the use of individual strains when considering biofertilizers formulation, and systemic protection may be associated to other metabolites different from IF.

The beneficial fungus <i>Piriformospora indica</i> induces fast root surface pH signaling and primes systemic alkalinization of the leaf apoplast upon powdery mildew infection <i>K. H. Kogel, F. Waller, A. Molitor, and H. Felle</i>	231
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Optimal defense in pine trees: constitutive and induced allocation of resin and polyphenolics in *Pinus radiata* 

Abstract: Optimal defense theory is based on the assumption that the within-plant allocation of defensive secondary metabolites is driven by the relative contribution to the overall fitness of particular plant tissues and their value in terms of costs. In this study, we examined the constitutive and methyl jasmonate-induced strategy of optimal allocation of the two major pine defenses, resin and polyphenolics, to two tissues with contrasting fitness value, stem and needles, along three parts of the plants (basal, medium and upper apical part) in *Pinus radiata* Don. seedlings. Induced responses of pine trees to methyl jasmonate were based on increased concentrations of total phenolics in leaves and resin compounds in the stem, but not significant changes were observed for phenolics in phloem either resin in needles. We observed a marked gradient of allocation within the plant, with different patterns between basal, medium and apical tissues of the pines. Resin content in the stem tissues was greater along an upward gradient. However, in leave tissues, both resin and phenolics content became greater along a downwards gradient. Our results indicate a marked pattern of allocation of defenses along the plant and among tissues relevant for plant fitness, which constitute the first confirmation of the Optimal Defense Theory for pine trees.

Impact of a natural elicitor for the biological protection of a major tropical crop:	
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# *Trichoderma atroviride* SC1 induces local and systemic resistance against grapevine downy mildew

Effects of elicitor treatment on the development of tomato	
and the interaction of root-knot nematodes	
S. Sanz-Alférez and R. Mochales	247
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**Abstract:** To determine if elicitors produced by biocontrol *F. sambucinum* (isolate FS-94) and *P. fluorescens* (strain 197) were able to induce spring wheat resistance against multiple pathogens, intracellular and extracellular elicitors of FS-94 as well as MF3-protein of 197 were used for treatment. The treatment of wheat seeds was conducted before their artificial inoculation with *F. culmorum* or seeds naturally infected with agents of root rots. Intracellular elicitors of FS-94 induced resistance in spring wheat against multiple fungi belonging to pathogenic root rot complex in Non-Chernozem zone of Russia (*F. culmorum*, *F. avenaceum*, *F. oxysporum*, *F. sporotrichioides*, *F. gibbosum*, *B. sorokiniana*). These elicitors decreased the incidence and severity of the diseases, reduced pathogen infectivity and suppressed sporulation of all tested root rot agents, except *F. avenaceum*. Extracellular elicitors of FS-94 elicitors prevented *Stagonospora nodorum* development on wheat leaves, while MF3 was active against *St. nodorum* only in combination with chitosan.

Effects of foliar nutrition on the reduction of diseases in extensive crops.

#### Field studies in Argentina

two on Zea mays, with a total of fourteen evaluation sites and four growing seasons (2005/2009). We monitored epidemics and yield in plots with or without foliar fertilizer application in production conditions in Argentina. The fertilizers under study were Nitrofoska<sup>®</sup> Foliar PS and Fetrilon<sup>®</sup> Combi. The results on soybean show a significant reduction of *Septoria glycines* levels and an increase in yield in plots treated with the foliar fertilizers. Median yields were 2600kg/ha, with a variation between 1900 and 3500kg/ha. With foliar nutrient applications, we obtained a median yield of 3204kg/ha, with the highest production at 4000kg/ha, representing improvements of 23%. In part, these improvements were due to a reduction in bacterial infections (Pseudomonas syringae pv glycines and Xanthomonas axonopodis pv glycines) because the crops with nutrient application showed leaves with better foliar structure, which decreased susceptibility to injury. The epidemiological monitoring on maize showed a reduction of symptoms caused by *Exserohilum* spp. and *Helminthosporium* spp. in the nutrient treated plots The best results in terms of final crop health were achieved in the treatments at the flowering period which, allowed yield improvements by 7-12%, entailing an increase of up to 1000 kg/ha. In the wheat crop the study object was leaf spots caused by Pyrenophora tritici-repentis and Septoria tritici. We compared nutrient application at stages 2.3-3.1 and 4.0-4.5 of the Zadoks developmental scale. Nutrient treatments resulted in yield improvement in all the evaluation sites. During dry seasons, the most favorable application time was at Zadoks stage 2.3-3.1, with improvements of 9-43%. In wet years the best application time was during Zadoks 4.0-4.5, with yield improvements up to 46% and reduction in disease severity of up to 60% compared to the untreated control. The results of these studies confirm the importance of foliar nutrition with micronutrients as a complementary tool in strategies of disease management to obtain a sustainable agro system.

#### Induction of systemic acquired resistance for the integrated management of TYLCD in greenhouse tomatoes

Antonella Sirigu, Francesco Chessa, Federico Corda and Mauro Nannini ....... 259-262 Abstract: The use of acibenzolar-S-methyl (ASM) for inducing systemic acquired resistance is a recommended practice for the management of several plant pathogens. During 2007-2008, three trials were carried out in commercial greenhouses in southern Sardinia (Italy) to evaluate the effects of this resistance elicitor on the spread of Tomato yellow leaf curl disease in protected tomato crops. In keeping with an integrated approach to crop protection, the efficacy of ASM was evaluated alone and in combination with non-woven row covers (NWRC) during the first weeks of the cropping period, a tactic adopted in recent years by a considerable number of growers on the Island. The results obtained in our experiments suggest that ASM may be a suitable means for TYLCD management. However its efficacy is strongly dependent on correct dosage and application frequency in relation to the actual dynamics of disease spread through the crop. The most interesting results were achieved when moderate infection progression was treated by integrating the use of non-woven fabric with early and frequent applications of ASM over an adequate period of time. The use of NWRC, by limiting disease pressure, creates more favourable conditions for the deployment of effective plant defence responses. However, the definition of IP&DM strategy based on the combination of these two tactics requires careful regulation of ASM applications not yet determined for TYLCD control.

### Induced resistance against powdery mildew in wheat -

#### a chance for less known inducers

changing. Untreated control demonstrated much more disease severity than the same cultivar treated by individual inducers. Disease severity after treatment with inducers was the lowest on BTH- and GI-treated and the highest on SA- and OB-treated plants. In 2008, when the disease incidence was extremely low, application of inducers resulted in diverse results. Preponderance of application dates except the first treatment by GB produced lower disease severity than CO. The earlier treatment by CU induced the lowest disease severity in comparison with other treatments. In general, the experiments showed long-term suppression of the powdery mildew severity on wheat.

## A Fusarium oxysporum extract induces resistance against *Botrytis* in pepper plants

Our approach was using an autoclaved extract of *Fusarium oxysporum* f. sp. *lycopersici* to induce plants. The plant roots were exposed to the extract and 48 hours later plants were challenged with a pathogen. Plant challenge was carried out in some plants on the leaves with the airborne fungus *Botrytis cinerea*. In other plants, the roots were challenge inoculated with the soilborne fungus *Verticillium dahliae*. The extract treatment controlled partially the infection of the leaves by the necrotroph *Botrytis cinerea* while it did not protect the plant against the fungus *Verticillium dahliae*.

Samples of the root and leaves were taken after the induction and after the infection for enzyme and gene expression assays. Peroxidase and chitinase activities were measured, but no changes were observed. The expression level of a set of genes related with resistance mechanisms was obtained through Real Time RT-PCR. All the genes were up-regulated by the FOL extract, both in the roots and the leaves.

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The efficiency of some bacteria as a microbiological control agent to cotton leaf worms in Egypt

were selected for further bacteriological studies. Preliminary characterization of the bacterial strains revealed that all strains were gram positive rod-shaped with variable morphology. Eight strains were identified as spore-forming species and two strains were gram-positive non-spore-forming rods. Some phenotypic tests (e.g., salt tolerance, heat tolerance, and some enzymatic activities) were done. The spore-forming rods were related to the genera *Bacillus* and *Paenibacillus*. The results pointed out that the bacterial strains in this study could be good candidates for biological control of the destructive cotton pests (leaf worms and spiny or pink bollworms) in Egypt.

Induction of systemic resistance of tomato to root-knot nematodes by

biogenic elicitors