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Induced Resistance – Chances and Limits

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INVITED SPEAKERS

Are population differences in plant quality reflected in the preference and performance of two endoparasitoid wasps?

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In recent years, increasing attention has been paid in exploring the role of direct plant defence, through the production of allelochemicals, on the performance of parasitoid wasps and their hosts. However, few studies have determined if parasitoids can detect differences in plant quality and thus preferentially attack hosts on which their progeny develop most successfully. In this study we examined the development and preference of two endoparasitoids, *Diadegma semiclausum* and *Cotesia glomerata*, developing in larvae of their respective hosts, *Plutella xylostella* and *Pieris brassicae*. In turn, these were reared on different wild populations of black mustard (*Brassica nigra*) originating in The Netherlands and Sicily (Italy), as well as single cultivated strains of *B. nigra* and brown mustard, *B. juncea*. The four mustard populations differentially affected development time and body mass of the herbivores and parasitoids. Contrasts among the means revealed significant differences mainly between *B. nigra* and *B. juncea*. Both parasitoids, however, preferred to alight on plants in which their progeny developed most successfully. In behavioural bioassays, *D. semiclausum* did not discriminate among the *B. nigra* populations and preferred to alight on *B. juncea*, which was the best plant population for parasitoid development. By contrast, *C. glomerata* females exhibited the lowest preference for Italian *B. nigra* populations, on which adult parasitoid size was the smallest. These results reveal that parasitoids can detect even small differences in plant quality presumably through their volatile blends and that plant preference and offspring performance in the two species are 'optimally synchronized'.

Oxylipins: a class of lipid signals mediating defence and plant development.

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JA is an essential hormone for the regulation of defence and developmental responses. It belongs to a family of active molecules, the oxylipins, that originate in the oxidation of various fatty acids usually by the activities of lipoxygenases (9-lipoxigenases and 13-lipoxygenases) and α-dioxygenases. Production of oxylipins and phytoprostanes, a group of non-enzymatically formed oxylipins, is a universal response of plants to pathogen attack. Given the importance of the 13-lipoxygenases in catalyzing the first step in the JA biosynthetic pathway, research has been largely devoted to 13-lipoxygenase expression and activity. However, in recent years interest in the role of 9-lipoxygenases and α-dioxygenases has revealed their participation in plant defence and developmental responses through the activation of specific signalling pathways. In *Arabidopsis thaliana* 9-lipoxygenase genes, *LOX1* and *LOX5*, express highly in root initials, and *lox1* and *lox5* mutants display enhanced root primordial emergence. A mutant, *noxy2*, that was defective in responding to the 9-lipoxygenase product, 9-hydroxilinolenic acid (9-HOT) (in a root-waving assay), was found not only to display alterations in root development, but also enhanced susceptibility to incompatible and compatible strains of *Pseudomonas syringae*, suggesting that a 9-lipoxygenase-derived oxylipin is both a modulator of root architecture and part of the defence mechanisms against pathogen attack. Accordingly, treatment of *Arabidopsis* seedlings with 9-HOT resulted in activation of molecular events common to development and defence responses. Further support to the participation of the 9-lipoxygenases in plant defence was obtained by characterization of additional *noxy* mutants showing enhanced susceptibility to biotrophic fungi.

Aspects of induced resistance against grapevine downy mildew

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Downy mildew, caused by the oomycete *Plasmopara viticola* is one of most detrimental diseases of grapevine (*Vitis vinifera* L.), a species deprived of natural resistance against this pathogen. Our lab and Goëmar laboratories collaborate on the implementation of oligosaccharide resistance inducers that are effective against grape diseases. Using a leaf disc screening procedure we identified, out of a dozen of compounds, oligogalacturonic acid, laminarin, and sulfated laminarin (PS3) as active resistance inducers against *P. viticola*, PS3 being the most effective one. In greenhouse tests using cuttings, PS3 reduced disease severity by 80%.
Examining defense reactions in grapevine revealed that PS3 treatment primed H_20_2 production, polyphenol deposition, and localised HR-like cell death, all these reactions being restricted to the cells invaded by the pathogen. Accordingly, up-regulation of defense genes was higher in inoculated treated plants than in simply treated ones.
Inhibitor assays indicated that PS3-IR was dependent on JA and callose deposition.
During the course of these studies we found out that *P. viticola* colonisation caused a heavy stomatal deregulation, that is stomata remained constantly blocked open, in an ABA insensitive manner. The origin of this previously unknown phenomenon still remains unclear.
In vineyard trials against downy mildew with PS3, results were quite unsatisfactory and the same holds true with a chitosane, though this compound exhibited 100 % efficacy in greenhouse trials. However, results with PS3 against powdery mildew were encouraging, though still variable. Understanding this puzzling discrepancy between controlled conditions and field results remains a challenging task. This requires to gain insight into the role of the various factors that influence IR expression in field conditions.

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

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Upon infection by a pathogen or upon treatment with some commercial fungicides plants can acquire resistance to a broad spectrum of pathogens and/or abiotic stresses. The acquired 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. However, the identity of such signaling components has remained elusive. We show that during development of acquired resistance in *Arabidopsis*, priming is associated with accumulation of mRNA and inactive proteins 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 acquired resistance. In addition, priming of defense gene expression and acquired 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 acquired resistance. The role of MPK3 and MPK6 in acquired resistance and the potential of priming for modern pest management in the field will be illustrated.

Beckers, G. J. M., Jaskiewicz, M., Liu, Y., Underwood, W. R., He, S. Y., Zhang, S., and Conrath, U. 2009. Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. Plant Cell, in press.
Prime-A-Plant Group (Conrath et al.) 2006. Priming: getting ready for battle. Mol. Plant-Microbe Interact. 19: 1062-1071.

The challenge of marketing *induced resistance-products (ISRP)* today

Cáceres, Sergio

Seaweed Canarias S.L.

Today, agro-businesses are facing the challenge of marketing a new generation of products based on ISR which probably constitutes the most valid alternative to overtake *old-fashioned* practices in agriculture. New EU regulations, competition, and market needs are part of a new agriculture in support of sustainability and environment, and this should be an ideal framework for the expansion of ISRPs. Relevant biotech companies have been investing for years in intensive R&D activities in order to provide new active compounds in line with ISR. However, despite its enormous potential, international distributors and fertilizer industries are having problems in taking one step ahead to establish ISRPs as a central part of their product portfolios. Moreover, a majority of final consumers appear reluctant to use ISRPs on a regular basis. In case you are interested in asking for a substitution of the chemical substances they have been using, which arguments would you provide in support of ISRPs? ISRP market segment is growing, but yet there is much to do. We expect markets will increasingly accept and commonly use induced resistance products in the future, but it will certainly take much longer than expected in case we follow the same path as today. We realize markets are not always ready to easily accept innovation, but continuous doubts about the efficiency of ISRP, lack of control by authorities, and bad practices from the industry are also part of the equation.

The role of the benzoxazinone pathway in aphid resistance in wheat

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The hydroxamic acids (HAs) are defence compounds biosynthesised in wheat that are active against a range of insect pests, pathogens and weeds. The most biocidal of these compounds, DIMBOA, is stored as the inactive glucoside within the plant vacuole and is released upon pathogen attack. We are testing the hypothesis that the relationship between HA production and resistance can be exploited, either through breeding or genetic engineering, to generate wheat varieties displaying environmentally sustainable resistance.

We have studied the localised expression of the *BX* (HA biosynthetic) genes, and compound accumulation in a range of wheat lines. Gene expression is highest in the coleoptile and root, but levels of DIMBOA (predominantly glucoside) are similar in the root and leaf. Transport or release of the compound from the roots may explain this. Gene expression and compound accumulation show limited genetic variation in hexaploid wheats but a diploid B genome wheat, *Aegilops speltoides,* produces high and sustained levels in the leaf. Tetraploid wheats contain higher levels than hexaploids, but less than *A. speltoides*, and diploid A genome wheats (*Triticum monococcum* and *T. boeoticum*) contain no HAs in the foliar tissue.

The effect of the cereal aphids *Sitobion avenae* and *Rhopalosiphum padi* on *BX* gene expression and HA accumulation has been investigated. The aphids do not affect *BX* gene expression but cause a rapid localised increase in plant glucosidase gene expression accompanied by conversion of inactive glucoside to free DIMBOA. Hence, aphid feeding induces the release of stored, inactive compound but not *de novo* biosynthesis. Preliminary screening indicates that there is genetic variation in the magnitude and kinetics of this response. DIMDOA levels are regulated in the plant leaf to prevent auto toxicity and attempts to increase free DIMBOA concentration by infiltrating the compound to cut leaves results in its immediate glucosylation. Therefore a means to enhance aphid resistance by exploitation of the HA pathway may involve both increase of the storage pool of glucoside and an increase in the inducibility of the glucosidase in response to aphid feeding.

Resistance induction and priming by plant volatiles

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Since the first reports on ‘talking trees’, descriptions of a defence induction in undamaged plants by air coming from infested neighbouring plants have been debated controversially. Later on, however, many studies demonstrated that volatile organic compounds (VOCs) that are released from a herbivore-damaged plant can

trigger defence responses in its neighbours. Field studies on lima bean demonstrated that plant-plant communication functions under ecologically realistic conditions: undamaged 'receiver' shoots suffered less from herbivory when they were exposed to the air that came from beetle-damaged emitters[1]. We now observed that plant-plant communication also affects the resistance of plants to pathogens. Lima bean plants became more resistant to bacterial with *Pseudomonas syringae* pv. syringae when they were growing close to conspecific neighbours in which pathogen resistance had been chemically induced with benzothiadiazole (BTH). Challenge infection after exposition to induced and non-induced plants revealed that the air coming from induced plants mainly primed resistance, as *pathogenesis-related protein* 2 (*PR*-2) was expressed at significantly higher rates in exposed than in non-exposed individuals when the plants then were infected by a bacterial pathogen. Negative effects on plant growth of BTH-treatment, as they regularly occur as a consequence of high costs of direct resistance induction, were not observed in the VOC-exposed plants. Volatile-mediated priming appears a highly attractive means for the tailoring of plant pathogen resistance.

Priming of defence genes by airborne signalling appears to be the rule rather than the exception, as VOCs also primed defence genes in corn[2, 3] and extrafloral nectar (EFN) secretion (an indirect defence against herbivores) of lima bean[4]. Priming prepares the plant to respond more rapidly and/or effectively to subsequent attack and can be activated at much lower concentrations of the resistance-inducing signal than full induction of active defences[5]. Given the fact that volatiles diffuse in the air, it is plausible that resistance-inducing volatiles are often diluted to priming concentrations. In fact, self-priming by herbivore-induced volatiles has been described in the context of airborne within-plant signalling[1, 6]. This suggests a two-step regulatory system in which airborne and vascular signals interact in order to achieve an optimized orchestration of the systemic defence response. In this model, airborne signals prime distal tissues to respond more efficiently to vascular signals or direct attack[7].

The major benefits of airborne within-plant signalling as compared to long-distance signalling via the vascular system appear that airborne signalling overcomes restrictions resulting from the plant's orthostichy, that airborne signals can move independently of the unidirectional flow in phloem and xylem, and that volatile signal molecules can reach distal plant parts faster than compounds that are transported through vascular tissues[7].

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Plant innate immunity: at the cell wall and beyond

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The cell wall provides a passive barrier against pathogens and pests, is a reservoir of antimicrobial compounds and a source of signalling molecules. During microbial infection cell-wall perturbations occur that lead to changes in gene expression. The characterization of plant resistance to necrotrophic pathogens has revealed the regulatory function of cell wall function in the control of *Arabidopsis thaliana* immune responses. Thus, mutants (e.g. *ern1/irx1*) impaired in Cellulose synthases (CESAs) required for the synthesis of cellulose from secondary cell wall shows a constitutive accumulation of a diverse set of antimicrobial compounds, which lead to a broad spectrum resistance to pathogens. Also, the accumulation of these antimicrobials has been found to control Arabidopsis resistance to non-adapted necrotrophic and biotrophic fungi. Biased resistance screenings of *Arabidopsis* cell wall mutants has been performed, and novel signal transduction pathways and regulatory proteins controlling plant innate immunity have been identified. Among them, several Receptor-Like-Kinases (RLKs), such as ERECTA, and a Mitogen-Activated Protein Kinase Kinase Kinase (MAP3K) were characterized. Plant RLKs have been suggested to be important components of the cell-wall monitoring integrity pathway, and to regulate basal resistance to pathogens by controlling the recognition of microbial patterns. MAP3Ks regulate RLKs-mediated innate immunity response by activating MAP Kinase cascades. The genetic interaction among these novel components of innate immunity will be presented.

SUBMITTED PAPERS

Chemically induced resistance to pathogens and mycorrhizal symbiosis in soybean: Reciprocal effects and impact on plant fitness.

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The aim of this work is to assess the impact of chemically induced resistance on beneficial plant-microbe interactions. We tested the hypothesis that soybean plants treated with a chemical resistance elicitor (Bion ®) are colonized by arbuscular mycorrhizal fungi to a lower extent than untreated plants and investigated whether this effect can be explained by the biochemical defence status of the plants.
Soybean seedlings were mycorrhized with *Glomus mosseae* (Gm) and *G. intraradices* (Gi). Non-mycorrhized seedlings (Nm) were used as controls. Six weeks after planting, half of the plants were sprayed with an aqueous Bion ® solution in order to induce systemic resistance to pathogens. Several plants of each of the six treatments (Gm induced, Gm not induced, Gi induced, Gi not induced, Nm induced, Nm not induced) were harvested one, two, and four weeks after the induction with Bion ® in order to check mycorrhization levels and to undertake biochemical and molecular analyses. The remaining plants were harvested at the end of their life cycle in order to analyse the production and quality of seeds to find out whether the induction of systemic resistance incurs fitness costs.
One week after the application of Bion ® the percentage of roots colonized by *G. mosseae* was significantly lower in induced than in control plants. This effect then levelled off, since it could not be observed in the plants harvested two and four weeks after the application of Bion ®. We conclude that the negative effect of the induction of systemic resistance on the mycorrhization of soybean plants by *G. mosseae* is only a transient one. In plants inoculated with *G. intraradices* the mycorrhization levels were low at the time of the chemical treatment, so the effect of the induction of systemic resistance could not be assessed.
The induction of systemic resistance increased significantly the concentration of proteins both in roots and leaves of Gm and Gi plants, but not in Nm plants. Chitinase activity was significantly greater in leaves of Gm and Gi plants after the induction of resistance, while no significant changes were observed in Nm plants. Mycorrhization with *G. mosseae* and *G. intraradices* could, thus, have a priming effect in soybean plants. The molecular analysis confirmed higher induction of pathogenesis-related gene expression upon Bion ® treatment in mycorrhizal plants.
Remarkably, the induction of systemic resistance did not incur fitness costs in soybean plants colonized with *G. mosseae*, while in those colonized with *G. intraradices* and in non-mycorrhizal plants the induction of resistance caused a significant decrease in the number of seeds per plant, the seed dry weight and oil content. Mycorrhizal association with *G. mosseae* can help soybean plants to compensate the fitness costs related to the induction of systemic resistance.

Impact of the presence of a generalist predator, the minute pirate bug *Orius insidiosus* (Hemiptera:Anthocoridae), on tomato induced defenses

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The induction of plant resistance to herbivory has been mostly studied as a response of the plant to direct, physical interactions with the herbivore, through behaviors such as feeding[1], ovipositing[2] or walking[3]. But little is known about how environmental, indirect clues of the proximity of herbivores may be exploited by the plant to ready its defenses against the impending threat of herbivory.
Here we explore how the presence of a generalist predator, *Orius insidiosus* (Say), may work as an early clue of the immediacy of herbivores in tomato (*Solanum lycopersicum*), even before herbivores actually initiate physical contact with the plant.
Using the technique of real-time PCR, we monitored how the presence of *O. insidiosus* impacted the level of induction of several defense genes associated to the jasmonic acid (JA), salicylic acid (SA) and ethylene activation pathways in young tomato leaves.

When a young tomato leaf is caged for 24 hours with *O. insidiosus* adults, we report a significant increase of the level of expression of some defense genes associated to the JA pathway, such as *arg* (arginase II), *pin2* (wound inducible proteinase inhibitor II), *ppoe* (polyphenol oxidase) and *dxs2* (2-deoxy-D-xylulose-5-phosphate synthase, involved in volatile emission). *Acgl* (acidic glucanase), a gene associated to the salicylic acid pathway, was also significantly induced. However no significant induction was observed for the protein kinase 1b (*tpk1b*), and an osmotin precursor (*osm*), both associated to the ethylene pathway.
The mechanisms by which the plant detect the presence of the predator are not known yet, but we observed that the density, life stage, gender and reproductive status impacted the level of defense gene induction. We will discuss our results with a particular emphasis on the possible ecological meanings of this plant-insect interaction.

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Some aspects of influence of exogenous methyl jasmonate on strawberry plants and on population size of two-spotted spider mite (*Tetranychus urticae* Koch.)

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In two experiments conducted on strawberry plants, the effects of exogenous methyl jasmonate (JA-Me) on population size of the two-spotted spider mite (*Tetranychus urticae* Koch.) as well as on the plant growth, and its flowering and yield were investigated. Experiments were conducted on strawberry plants of Kent cultivar growing in glasshouse conditions. In the first experiment, a negative influence of the JA-Me treatment of plants on the population growth of the two-spotted spider mite was observed. The second experiment had been carried out for two years. In the first year of the study, all flowers of the strawberry plants were picked up and no effect of JA-Me treatment on total leave area of the plants was observed. However, in the second year of the study, when plants were allowed to flower and fruit, reduction of the plant growth was stated after JA-Me treatment. No influence of JA-Me was observed on flowering and yield of strawberry plants.

Priming plant innate immunity

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Specific environmental stimuli can prime the plant's innate immune system to express an augmented defence reaction upon subsequent attack by insects or pathogens. Priming is a cost-efficient defence strategy that improves plant fitness under stress-full conditions. We investigate priming against pathogen infection in Arabidopsis upon application of the xenobiotic compound beta-aminobutyric acid (BABA). This Arabidopsis – BABA model system has allowed us to identify genetic and epigenetic mechanisms behind priming of salicylic acid-dependent defence, as well as a novel regulatory gene during the onset of priming of cell wall-based defense against fungi and oomycetes. In addition, our research is focused on defence priming against herbivorous insects in maize, which can be triggered by herbivore-induced volatiles or below-ground infestation by insects. This research points to novel airborne priming signals that act in synergy with green leaf volatiles to shape the plant's JA-dependent defence reaction. We also discovered that below-ground infestation by root herbivores primes above-ground tissues for augmented induction of defence metabolites.

An array of responses to insect feeding in cabbage cultivars

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In their natural surrounding, plants are constantly attacked by all kinds of herbivorous insects. To be able to survive, plants have developed a wide spectrum of defense strategies that can be constitutively present or activated upon herbivore attack. Transcriptional profiling after herbivore feeding reveals, at the molecular level, how plants respond to this type of stress. Differences in transcriptional profiles often underlie phenotypic variation among plants from the same species. Studying intraspecific variation on the molecular and the ecological level in an integrated way provides insight into plant defense mechanisms. Intraspecific variation in resistance or susceptibility to herbivores has been studied through bioassays. However, few studies link this with a genome-wide transcriptional analysis. We take such an approach to study the interaction between white cabbage (*Brassica oleracea* var. *capitata*) cultivars and either the caterpillar *Pieris rapae* or the aphid *Brevicoryne brassicae.* As *Brassica* full-genome microarrays are not yet available, 70-mer oligonucleotide microarrays based on the genome of *Arabidopsis thaliana* were used.
We show that there is intraspecific variation between *B. oleracea* cultivars with respect to herbivore performance of both *P. rapae* and *B. brassicae* in the greenhouse as well as in the field. The transcriptional responses after 24, 48, and 72 hours of *P. rapae* feeding on two cultivars that supported different herbivore performance showed variation in timing and regulation of individual genes. In contrast to *P. rapae*-induced plant responses, *B. brassicae* feeding resulted in the differential regulation of only a small number of genes in the two *B. oleracea* cultivars. The genes that were differentially regulated in response to aphid infestation were highly cultivar-specific.
We also monitored herbivore population dynamics and analyzed transcriptional profiles of the two *B. oleracea* cultivars in the field. In contrast to greenhouse conditions, field-grown plants face many biotic and abiotic challenges. Later in the season, clear differences in herbivore communities and transcriptional profiles were found. These differences are, at least partly, reflected in differences in expression levels of particular genes.
In conclusion, the data show that intraspecific variation among plants has a strong impact on their interaction with herbivores, both at the molecular and the ecological level.

Model plants as tools to study the impact of fungal root endophytes on plant-pathogen interactions

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Root endophytic fungi are able to modulate the interaction between plants and pathogens. This can lead to an induced resistance against pests and parasites, but might also enhance the susceptibility of the hosts. In order to ensure a positive outcome of the application of such endophytes, it is necessary to understand the processes and the mechanisms behind in such tripartite interactions. If all modern tools of experimental biology should be used for this purpose, it is advantageous to use model plants, where many data are available and genetical and molecular methods can be easily applied.
Fungi of the phylum Glomeromycota form arbuscular mycorrhiza with the roots of 80% of terrestrial angiosperms. Besides supplying the plant with mineral nutrients, they increase the resistance against root pathogenic fungi. In order to analyse mycorrhiza-induced resistance of legumes against the oomycete *Aphanomyces euteiches* we chose *Medicago truncatula* as a model. Screening an array with 16,000 plant genes resulted in hypotheses concerning the hormone biosynthesis and perception, the involvement of particular elements of signal transduction chains, the metabolism of reactive oxygen species and the biosynthesis of phytoalexins.
Piriformospora indica belongs to the Sebacinales (Basidiomycota). The endophyte infects the roots of many plants and grows in the cortex, where it colonises dead plant cells. It has been shown that *P. indica* induces resistance in barley against root and shoot pathogens, and that antioxidants might be involved in this phenomenon. We conducted a series of experiments for revealing the conditions for application of this root endophyte in hydroponic cultures of tomato. Analysis of its impact on the interaction of plants with viruses showed a light-dependent increase of resistance or susceptibility. First results of gene expression analyses will be presented.
Tomato was also used to screen new root fungal isolates for their potential of being used as biological agents. This has led to the identification of a group of Ascomycota which show various influences on plant development and health. Among those, three increase tomato biomass and one seems to induce the resistance against *Verticillium dahliae*. These new endophytes and their interactions with plants are currently being characterised using different experimental systems.

Root herbivore induced shoot resistance- physiological explanations for a counterintuitive phenomenon

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Root herbivore attack can have a profound systemic impact on aboveground plant physiology. It has been shown repeatedly that root herbivory increases shoot resistance, yet the underlying mechanisms and potential evolutionary significance of this phenomenon have remained unclear. We therefore investigated how the belowground-feeder *Diabrotica virgifera* influences aboveground physiology and resistance of *Zea mays*.
Leaves of *D. virgifera* infested plants displayed increased resistance against the herbivore *S. littoralis* in the laboratory as well as against lepidopteran pests the field. This effect coincided with a profound reconfiguration of the plants' aboveground physiology and defense status: Leaves of root herbivore attacked plants showed increased concentrations of abscisic acid (ABA), ABA-dependent defense gene expression as well as higher concentrations of the defensive metabolite 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA). They were furthermore primed for the expression of the phenolic compound chlorogenic acid. Our experiments suggest that many of these responses are the result of a highly efficient feedingy strategy of *D. virgifera* that upsets the plant's water balance.
To answer the question if the increased resistance in the leaves is the result of the activation of ABA-dependent leaf-defenses, we inhibited ABA biosynthesis chemically by using sodium tungstate and genetically via antisense and sense expression of *Zm-NCED*. Furthermore, we manipulated the root-herbivore induced loss of leaf water by varying soil moisture. The results show that *S. littoralis* is strongly influenced by the plant's water status, but not by ABA. We conclude that *D. virgifera* induces shoot resistance against *S. littoralis* by triggering ABA-independent changes in the plant's water balance, a phenomenon that is most likely a physiological constraint resulting from the interaction between the root herbivore and its host plant.

Molecular analysis of P. indica-induced resistance in barley and Arabidopsis: the role of plant hormones and host cell death

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The mutualistic basidiomycete *Piriformospora indica* colonizes a broad range of monocot and dicot plants thereby transferring various beneficial properties to its host. This broad host range indicates that the fungus has developed efficient strategies to manipulate innate immune responses and metabolism in different plants. It was the aim of the present study to identify plant compatibility factors that are required for successful root colonization. A novel transcript subtractive hybridization assay identified several differentially regulated genes known to be involved in stress responses, phytohormone- and secondary metabolism, autophagy, and protein processing. Up-regulated among others were the gene encoding S-adenosylmethionine synthase serving as methyl donor for the synthesis of ethylene and the structural gene for S-adenosyl methionine decarboxylase proenzyme (ADC) required for synthesis of spermine and spermidine. *De novo* synthesis of ethylene upon root colonization was verified by analysis of the ethylene precursor 1-aminocyclopropane 1-carboxylic acid (ACC) in barley and by monitoring GUS expression with ACC-synthase and ADC gene promoters in *Arabidopsis*. Mutants of *Arabidopsis* that enhance ethylene synthesis promoted root colonization, while mutants impairing ethylene perception showed a 20% reduction of infestation. Mutants that decrease polyamine synthesis lowered root colonization by *P. indica* with 18-27%. In conclusion, ethylene und polyamines appear to be general compatibility factors recruited by the fungus to colonize a multiplicity of host plants.

Glucosinolates as a major factor in disease resistance of Arabidopsis

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The phytoalexin (camalexin)-deficient Arabidopsis mutant *pad2* is highly susceptible to the oomycete pathogen *Phytophthora brassicae* (Plant J 49, 159 (2006)). However, the deficiency in camalexin accumulation was shown not to be the cause of this susceptibility. The *pad2* mutant was found to also accumulate less glucosinolates (GS) in response to insect herbivory (Plant J 55, 774 (2008)). Consequently, we have addressed the question whether or not the GS deficiency of *pad2* can explain its susceptibility to *P. brassicae*. Transcript profiling revealed that biosynthetic genes of indole-glucosinolates (IGS) are up-regulated in response to *P. brassicae*. Interestingly, the double mutant *cyp79B2/cyp79B3*, with compromised camalexin and IGS biosynthesis is highly susceptible to *P. brassicae*. Hence, the susceptibility of *pad2* can be explained by the combined deficiency of secondary metabolites. The genetic data are supported by direct inhibition assays showing that IGS and camalexin inhibit the *in-vitro* growth of *P. brassicae*. Analysis of infection kinetics argues for cell death-independent role of IGS early in the infection process and a late, less important role for camalexin. Together our results indicate that the IGSs, previously known as components of anti-herbivore defence, play a major protective role towards *P. brassicae*.

Networking by small-molecule hormones in plant immunity

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Plants live in complex environments in which they intimately interact with a broad 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 highly 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[1]. 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.

The plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) emerged as key players in the regulation of the induced defense signaling networks involved. SA- and JA/ET-dependent pathways regulate defense responses that are differentially effective against specific types of attackers[2]. Pathogens with a biotrophic lifestyle are generally more sensitive to SA-dependent responses, whereas necrotrophic pathogens and herbivorous insects are commonly deterred by JA-dependent defenses. Global expression profiling of pathogen- and insect-induced Arabidopsis wild-type and signaling mutants highlighted substantial cross-talk between the SA and JA signaling pathways, often resulting in antagonistic effects on plant defense[3]. This pathway cross-talk is thought to provide the plant with a powerful regulatory potential that helps deciding which defensive strategy to follow, depending on the type of attacker encountered. Using a pharmacological approach to dissect the kinetics and mechanisms underlying SA/JA cross-talk, we demonstrated that the SA-mediated antagonistic effect on JA-responsive gene expression is conserved among Arabidopsis accessions, highlighting the importance of this mechanism for plant survival[4]. The kinetics of SA and JA signaling appears to play an important role in the outcome (antagonistic, synergistic, neutral) of the SA/JA interaction. The antagonistic effect of SA on JA-responsive gene transcription appears to be linked to SA-induced changes in the cellular redox potential, suggesting that SA/JA cross-talk is redox regulated. Several key regulatory proteins involved in pathway cross-talk have been identified[3]. For instance, NPR1 was shown to function as a modulator of cross-talk between SA and JA[5]. Currently, our research is focused on the mode of action of SA/JA cross-talk and the identification of potential targets in the JA signaling pathway through which SA can antagonize JA-dependent defenses. Furthermore, we are interested in how pathway cross-talk affects induced resistance against pathogen and insects.

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Potential benefits and limitations of grapevine self protection induced by certain beneficial microbes

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Some beneficial soil-borne microorganisms can promote plant growth and reduce disease symptoms through activation of the induced systemic resistance (ISR) in plants. ISR enhances plant resistance against various types of pathogens or insects, and it can be regarded as a promising biocontrol strategy. However, scarce information is available on the efficacy, persistence, and fitness cost of ISR in non-model plants.
We analysed the systemic resistance against *Plasmopara viticola* activated in grapevine by the biocontrol agent *Trichoderma harzianum* T39 in comparison to resistance activated by benzothiadiazole (BTH). T39 activated a systemic plant-mediated resistance and reduced downy mildew symptoms at a level comparable to treatments with BTH, a known activator of systemic resistance mediated by salicylic acid. Treatments of basal leaves or leaves on one side of the shoot homogeneously activated disease protection in untreated leaves, independently of position on the shoots, whereas root treatments did not induce resistance in leaves. However if only the treated leaves were considered, T39 induced a lower protection level and a shorter persistence of the effect compared to BTH. Repeated BTH treatments entailed energy costs, which strongly reduced grapevine growth, probably because of the allocation of metabolic resources into defense mechanisms. In contrast, repeated T39 treatments did not affect photosynthesis and plant growth (number of leaves and leaf area, fresh and dry weight of leaves, shoots and roots). These results suggest the activation of different defense pathways in grapevine after BTH and T39 treatment.
Expression of some marker genes involved in different plant resistance mechanisms was analyzed by real-time RT-PCR in untreated and T39 or BTH treated plants, before and after pathogen challenge. Surprisingly, in absence of the pathogen BTH treatments did not induce *PR-1*, *PR-2, PR4* and *LOX9* expression in local and systemic leaves and similar results were obtained with T39 treatment. However, a significant induction of the defense genes was detected 24 h after pathogen inoculation. No priming effect was observed in the BTH- and T39-treated plants inoculated with the pathogen.
To identify key genes involved in grapevine self protection induced by T39, we intend to undertake a broader analysis by measuring the gene expression of all the grapevine transcripts under different treatment conditions.

Mobilization of Insect Defenses in Maize

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We have been investigating the factors that lead to the mobilization of a novel cysteine protease, Mir1-CP and other resistance proteins that accumulate in the whorls of insect resistant maize genotypes in response to fall armyworm (FAW, *Spodoptera frugiperda*) feeding. The first component of this pathway is FAW saliva, which appears to induce the accumulation of transcripts encoding chitinases, maize protease inhibitor and ribosome-inactivating protein 2 in the whorl. Surprisingly, Mir1-CP accumulates at the feeding site within 1 hour of caterpillar feeding whereas its transcript levels do not increase until 24 hour after feeding begins. In addition to accumulating directly at the wound, Mir1-CP also accumulated several cm distal from the feeding site. Because this suggested that Mir1-CP might move systemically, we determined Mir1-CP's cellular localization. Immunogold localization with silver enhancement indicated that Mir1-CP was present in plastid-like inclusions in the thick-walled sieve elements in the whorl. Mir1-CP was also detected in the vascular tissue of maize roots and its levels there increased 24 hours after herbivore feeding in the whorl. Furthermore, when roots were removed from the plants prior to FAW infestation, the levels of Mir1-CP that accumulated in the whorl were significantly lower. Hence it appears that FAW feeding in the whorl enhances Mir1-CP accumulation in the root, which may in turn,

be transported to the whorl.
We have demonstrated that Mir1-CP attacks and permeabilizes the peritrophic matrix (PM) of many caterpillar pests by degrading PM proteins. Its LC_{50} values range from 0.8 to 8.0 ppm depending on the pest. Because we unexpectedly found Mir1-CP in the roots, we speculated that it also could protect plants from root feeding insects such as the western corn rootworm (WCRW, *Diabrotica virgifera*). To test this, resistant (Mp708) and susceptible (Tx601) maize inbreds were infested with WCWR larvae. Only 5 larvae were recovered from the resistant genotype while 35 were recovered from the susceptible genotype, and WCRW larvae recovered from resistant plants weighed approximately 50% less than those recovered from susceptible plants. The mechanisms causing this dual insect resistance are being elucidated.

Sugar signaling as a new way for vegetable and fruit induced resistance against insects, pathogens and nematodes

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By spraying soluble carbohydrates on the leaves of maize, tomato, potato, bean plants and apple trees we could induce resistance to pests and diseases. Above and below ground resistance mechanisms could be observed against pests and pathogens attacking different plant parts (i.e. leaves, fruits and roots). Experiments conducted in growth chambers, glasshouse and semi-field situations showed dose effects of soluble carbohydrates (sugars) at concentrations in the order of 0.01-100 ppm (0.01 to 100 mg / litre). Variation in induced effects were observed, linked to the sugar, its concentration, mode of application and plant species. One hexose sugar consistently had a more general effect on all the plants species, pests and diseases tested. The possible resistance mechanisms observed involve antixenosis against Lepidoptera and nematodes; i.e. interference with host acceptance effective before attack. In addition, effects were observed on leaves of tomato and bean plants against the fungal pathogen *Botrytis cinerea*. Low dose foliar sprays and soil drenches also induced resistance in roots against several nematode species attacking tomato, potato and vegetable crops. The inducible effects following sugar and specific sugar analogue applications complemented constitutive resistance to the potato cyst nematode *Globodera rostochiensis* in a partially resistant potato cultivar.
Applications of the technology in apple tree orchards and a garden orchard over three years gave protection against its main insect pests *Cydia pomonella* (Lepidoptera). The induced effects showed an efficacy of the sugar treatment alone and also improvements in the efficacy of chemical (standard pesticides) or biological control. This indicates a practical use for the inducible resistance in Integrated Pest and Diseases Management, particularly when currently used pesticides are being withdrawn by the EU, USA and other countries.
Knowledge of leaf cuticular permeability to these sugars, sugar signalling in plants, sugar treatment effects on plant growth, on primary metabolites, durations and delays of effects, insect behavioural response to the changed ratio of primary metabolites on the leaf surface, provide information leading to some hypotheses on the induced resistance mechanisms involving both antixenosis, antibiosis, increased plant vigour and related stress tolerance.

Perception of Herbivores in the Tomato Plant

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Although it is becoming increasingly appreciated that leaf surface traits such as trichomes may be as important determinants of herbivore performance as internal leaf chemistry, the role of trichomes in plant defense signaling has not been reported. Here we demonstrate that the rupture of tomato foliar glandular trichomes by caterpillar or moth contact induces the expression of defense genes (e.g., proteinase inhibitor 2 =*pin2*) regulated by the signaling compound jasmonic acid. Neither insect chewing nor the release of salivary components is required to initiate this induced response. Genes encoding proteins involved in jasmonic acid biosynthesis are expressed in glandular trichomes. Using tomato mutants, we confirmed that both glandular trichomes and jasmonic acid are required for the contact-induced expression of *pin2*.

Additionally, H_2O_2 formed at the leaf surface is required to elicit *pin2* expression. Because these defenses would be activated prior to egg hatch, this early detection system for herbivores may be of considerable significance.

Differential responses of herbivorous insect and acari species to a protease inhibitor family from barley

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The aim of this study has been to assess the potential effects as defence proteins of the whole protease inhibitor gene family from barley on arthropod pests. This family comprises 13 genes encoding proteins, cystatins (HvCPI-1 to HvCPI-13), able to specifically inhibit cysteine-proteases of the papain C1A and legumain C13 classes from plants. Besides their function as endogenous regulators of the protein turn-over, cystatins can play a role in defence due to their capability to inhibit proteases from heterologous pests and pathogens. In addition, the protective action of the barley cystatins is also supported by their induction of some of them in response to arthropod damage, wounding and after treatments with salicylic and jasmonic acids.
The *in vitro* inhibitory activity of the 13 cystatins, purified as recombinant protein from *E. coli* cultures, was tested against five species of phytophagous arthropods with cysteine protease activity, mainly located in their guts: two aphids, *Myzus persicae* and *Acyrthosiphon pisum*, two coleopteran *Leptinotarsa decemlineata* and *Diabrotica virgifera* and one acari, the two-spotted spider mite *Tetranychus urtica*e. Enzymatic activities present in these arthropods were inhibited by most of the barley cystatins, being cathepsin L-like the preferential target of these proteins. It resulted particularly efficient the recombinant protein HvCPI-6 able to drastically reduce cathepsin-L and B-like activities in the insects and acari tested.
Bioassays using the two aphids reared on artificial diets supplemented with the HvCPI6 cystatin revealed a strong increase in the mortality rates of *A. pisum* while *M. persicae* was not affected. However, clear changes although different, in the cysteine proteases activity of both aphids are detected and support the different impact of the cystatins in each species.
The *in vivo* effects of transgenic plants over-expressing the barley cystatin on the arthropods tested are currently being analysed.

Arbuscular mycorrhiza confers systemic resistance against gray mold (*Botrytis cinerea*) in tomato through priming of JA-dependent defense responses

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Arbuscular mycorrhizal fungi (AMF) are soil fungi that form mutualistic symbioses with the roots of about 80% of all terrestrial plants, including most agricultural, horticultural and hardwood crop species. The association results in improved plant fitness in terms of nutrition and resistance to biotic and abiotic stresses. In addition to the well-known protection against root pathogens, diverse studies have shown a protective effect of the symbiosis against shoot pathogens [1].
In previous experiments we evidenced that mycorrhizal plants are more resistant to the necrotrophic fungus *Botrytis cinerea*, causal agent of gray mold in tomato. Symptom development in whole plants upon spray inoculation with *Botrytis* was significantly reduced in tomato plants colonized by the AMF *Glomus mosseae*. In order to obtain an easy and reproducible method to evaluate disease development, we have established a detached leaf assay. We found that the diameter of spreading lesions was significantly smaller in leaves from mycorrhizal plants than in those from non-mycorrhizal ones. These results confirm that mycorrhiza confers systemic resistance to *B. cinerea*.
It has been proposed that priming for jasmonate (JA) dependent responses plays a key role in the induction of resistance by beneficial microorganisms [2, 3]. We have confirmed that mycorrhizal plants display a potentiated response to exogenous application of JA and to wounding. Remarkably, the induction of expression of JA-

dependent marker genes in response to *Botrytis* inoculation is higher in mycorrhizal plants. The induction of JA dependent defenses in tomato confers resistance to *Botrytis* in tomato plants (Vicedo and Flors, personal communication). Thus, our results support that systemic resistance to *B. cinerea* in mycorrhizal plants is associated to priming of JA-dependent responses. The possible role of the peptidic hormone systemin in the regulation of the priming phenomena associated with mycorrhiza-induced resistance will be discussed.

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Priming and pathway in the biosynthesis of volatile organic compounds in melon plant induced by FEN560 novel plant extract elicitor

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This study describes the priming of induced resistance in melon plant treated by FEN560, a plant extract elicitor exctracted from Trigonella foenum-graecum. FEN560 is developed by the laboratory of Agro-ressources and Biological and Industrial Processes (University of Montpellier II, France) in collaboration with SOFT-Company (Port la Nouvelle, France). In order to assess resistance induced after FEN560 pre-treatment, two cultivars of melon plant were artificially infected with Fusarium oxysporum Schlecht. f. sp. melonis race 1 (FOM 1). The chosen cultivars have different levels of resistance to FOM1. As marker of induced resistance, changes in enzymatic activities of following enzymes were noted: lipoxygenases (LOX), and peroxidases (POD) in addition volatile organic compounds (VOC) emission were also monitored to evaluate the induction of resistance and the priming phenomenon. Two methods of treatment were tested to evaluate the systemicity of induced resistance triggered by FEN560. Treatments were performed either by spraying of the FEN560-solution or through irrigation. Enzymatic kinetics were measured by spectrophotometry. Besides this, comparative study of POD isoenzyme in roots and cotyledons was also performed by iso-electro-focusing (IEF). Assessment of the VOC in cotyledons and roots was performed by Headspace Solid Phase Micro-extraction (HS-SPME) and screened by gas chromatography coupled to mass spectrophotometry. In the second part of this study expression profiles of following genes were studied: NPR1, PR1, LOX (9-LOX and 13-LOX) and POD (APX-P and APX-C). To this end data has been accumulated and still being analyzed.
Results of LOX and POD kinetics activities and their isoforms as well as the emission of the VOC show that the FEN560 markedly induce the resistance in both melon cultivars. However qualitative and quantitative differences of induction were observed between the two cultivars as well as in organs of the same seedling. Activation of the enzymatic markers and the emission of VOC also depends on the mode of treatment. Indeed, the induced resistance triggered by the foliar treatment is similar to Systemic Acquired Resistance (SAR) while the induced resistance triggered by the treatment of roots is comparable with Induced Systemic Resistance (SIR). After foliar treatment the priming is localised mainly in susceptible cultivar on infection sites (second elicitation). It results that whole plant immunity is activated and that induction relates to the secondary site of aggression. The study of VOC emission shows an increase in the emission of aldehydes and alcohols. Most important of them are: 2-hexanal et le 2,2-dimethyloctenol. In addition, new compounds were detected such as: octenol, hexadecenol, 1-hexanol-ethyl. These variations were correlated with increased LOX activities seen after the induction of resistance. An important emission of terpenes and ketones were also observed such as limonene, eucalyptol and linalool. New molecules are also observed in primed plants such as β & α ionone, 4-oxoisophorone and δ-2-decalactone. The newly detected VOC in primed plant can be another signature of priming phenomenon.

Implications of nitrogen metabolism in plant basal resistance and priming

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A mutation in a high affinity nitrate transporter (HATS) codified by *NRT2.1* conferes enhanced resistance against *Pseudomonas syringae* by priming SA dependent responses. Interestingly, *nrt2.1* mutation is not associated to different nitrate levels in normally fertilized plants. In addition, mutant plants showed altered ABA control by *Pst*. ABA level does not change in *nrt2.1* 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 *nrt2.1*. Therefore it seems that *nrt2.1* displays some interference with ABA signaling, which probably results in a deficient ABA control by the pathogen. This establishes a possible link between nitrate transporters and plant responses to biotic stresses.

Implication of callose deposition in the OCP3-mediated disease resistance to necrotrophic pathogens

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The phytohormones abscisic acid (ABA) and methyl jasmonate (MeJA) control the signaling pathways responsive of the plant adaptive responses to drought and pathogenic fungi infections, respectively. OCP3 is a transcriptional regulator of the Homeobox family and that has been previously demonstrated to be required for mediating specific aspects of plant responses as mediated by both ABA and MeJA. The *ocp3* loss-of-function mutant shows a JA-dependent enhanced resistance towards *Botrytis cinerea* and *Plectosphaerella cucumerina* that is accompanied by an enhanced drought tolerance and increased sensitivity to ABA. This phytohormone has been proposed to be involved in the callose deposition surrounding the infection sites to restrain pathogen entry. In this work we show that the enhanced resistance of *ocp3* plants towards *B. cinerea* and *P. cucumerina* goes along with an early and drastic increase of callose accumulation. These results suggest that OCP3 is implicated in the regulation of the rapid callose deposition involved in plant-pathogen interaction. To further study this observation we have generated a battery of mutants which allowed us to dissect the genetic requirements of the OCP3-mediated resistance towards necrotrophic fungi and the role played by ABA and JA in mediating the observed deposition of callose.

Using short-lived radiotracers to study short-term induced changes in resource dynamics.

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As sessile organisms, plants have evolved a wide arrange of defense mechanisms in order to reduce the impact or withstand enemy attacks. Upon damage, herbivores are confronted by the production of chemical compounds or physical structures by their host. This may reduce herbivore preference or performance through reduced tissue digestibility or/and increased tissue toxicity. However, plants can employ an alternative, not mutually exclusive, strategy in response to damage. Through so-called tolerance mechanisms, plants can undergo changes in their primary metabolism allowing them to fine-tune allocation of new and existing resources and temporarily direct them to plant organs where they are less accessible for the attackers.
One of the main aims of this project is to investigate short-term resource allocation in tomato and tobacco in response to simulated herbivory. We make use of short-lived radiotracers (^{11}C and ^{13}N) to study the distribution *in planta* of newly incorporated $^{11}CO_2$ and $^{13}NH_3$, avoiding the use of intrusive techniques and obtaining measurements in real time.
We applied Methyl Jasmonate (MeJA), a known defense elicitor, on the foliage of tomato plants and monitored carbon and nitrogen dynamics for 2 hours after radiotracer application. Shortly after the MeJA treatment (4hrs), we observed an

increase in carbon and nitrogen export out of treated leaves. Young developing leaves (apex) and roots are the strongest sinks for resources. After MeJA treatment, we observed an increase in labeled N in the root area as compared to the apex. There was also a trend showing an increase in carbon towards the roots after MeJA treatment. These results suggest an increase in root sink strength in response to simulated herbivory. $^{11}CO_2$ fixation was reduced in response to MeJA, hinting at a possible trade-off between growth and induced resistance. Overall, these results are in agreement with our hypotheses, showing a change in resource partitioning after simulated herbivory reducing the chance of resources being lost to herbivores. The study of short-term resource dynamics at a whole-plant level provides an useful insight that will allow for a better understanding of defense and tolerance mechanisms.

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Transcript profiling of plant-aphid interactions in barley as an approach to identifying resistance genes

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The bird cherry-oat aphid (*Rhopalosiphum padi* L.) is an important pest on cereals causing plant growth reduction without specific leaf symptoms. Wild barley has been used for resistance breeding, with reduced aphid growth as a resistance indicator. Wild barley and breeding lines causing up to 43% reduction of growth are available.

With the idea that at least part of the resistance is caused by induced resistance mechanisms, we performed microarray analysis of gene expression after aphid infestation in two susceptible and two partially resistant genotypes. One of the four lines with intermediate resistance is a descendant of two of the other genotypes, of which one is susceptible and the other resistant.

There were large differences in gene induction between the four lines, indicating substantial variation in response even between closely related genotypes. Genes induced in aphid-infested tissue were mainly related to defence, primary metabolism and signalling. Only 24 genes were induced in all lines, none of them related to oxidative stress or secondary metabolism. Few genes were down-regulated, with none being common to all four lines. Comparing gene induction in resistant and susceptible genotypes, five gene sequences were identified specifically induced in the resistant lines. Results from control plants without aphids also revealed differences in constitutive gene expression between the two types of lines. The gene sequences identified are now being examined for their expression in breeding populations characterized with regard to *R. padi* resistance and to the presence of a QTL for aphid resistance.

The study shows that the use of both near related and unrelated genotypes in a microarray comparison limits the number of putative genes for further investigation to a relatively small number. Chances of identifying resistance-related genes are high since both constitutive and induced candidate gene sequences and markers for QTL are tested in barley breeding populations segregating for the aphid resistance.

Role of the *Thctf1* transcription factor of *Trichoderma harzianum* in 6-pentyl-2*H*-pyran-2-one production and antifungal activity

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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-2*H*-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 wild-type, observing that the *Thctf1* 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 disruptants, in the absence or presence of a pathogen. A tomato microarray approach supports the changes in the plant transcriptome during these interactions.

New insights into the complex role of ABA in *Arabidopsis thaliana* resistance to necrotrophic pathogens

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Plant resistance to necrotrophic pathogens depends on the interplay of different signaling mechanisms, such as those mediated by the hormones SA, JA and ET. In addition to these well-characterized pathways, other plant hormones are emerging as novel regulators of plant resistance to pathogens. The role of ABA signaling in the regulation of plant innate immunity is complex and still not well understood. In Arabidopsis resistance to necrotrophic pathogens, such as *Plectosphaerella cucumerina*, a positive effect of the constitutive activation of ABA pathway has been described (Hernandez-Blanco *et al.* 2007). However, ABA biosynthetic (*aba1*) and signaling (*abi1*) defective mutants were found to be more resistant to necrotrophs than wild-type plants, suggesting a negative role of ABA in the regulation of plant resistance to this type of fungi. Comparative transcriptomic analyses of *aba1* mutant and wild-type plants upon *P. cucumerina* infection led to the identification of some defensive responses that were constitutively activated in the *aba1* mutant. Meta-analysis profiling demonstrated that *aba1* up-regulated genes were predominantly regulated by JA and SA. In addition, genetic analyses demonstrated that JA biosynthetic pathway is necessary for full resistance of *aba1* mutant to necrotrophs. Recent advances on the crosstalk among ABA, JA, SA and ET signaling pathways in the regulation of Arabidopsis immune response to necrotrophic fungi will be presented.

Hernandez-Blanco, C., *et al* (2007). Plant Cell 19, 890-903.

Cloning and characterization of a mutant impaired in *β*-aminobutyric acid-induced priming for cell wall defense in Arabidopsis

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The xenobiotic compound *β*-aminobutyric acid (BABA) primes Arabidopsis innate immunity against attack by microbial and herbivorous insects. This defence priming results in a faster, stronger and probably more sustainable defense reactions once the plant is exposed to pathogen or insect attack. BABA-induced protection against the oomycete *Hyaloperonospora arabidopsidis* is partially based on priming of salicylic acid (SA)-dependent resistance. However, most of this protection depends on an additional, SA-independent priming of early-acting cell wall defense. This priming results in augmented deposition of callose-containing papillae at the sites of pathogen attack and requires intact abscisic acid (ABA) signaling. In order to further dissect the pathways controlling SA-independent priming of cell wall defense, we screened an ethylmethane sulfonate (EMS)-mutated population of SA non-accumulating NahG plants for mutants impaired in BABA-induced immunity (*ibi*) against *H. arabidopsidis*. One of these mutants, NahG *ibi1-1*, fails to express BABA-induced priming of cell wall defense against *H. arabidopsidis* Treatment of NahG *ibi1-1* with

SA-analogue benzo(1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) induced resistance against *H. arabidopsidis*, indicating that the SA response pathway is unaffected in this mutant. Furthermore, NahG *ibi1-1* expressed wild-type levels of ABA-induced seed dormancy, suggesting that it is not impaired in ABA signaling either. Finally, we found that NahG *ibi1-1* accumulates excessive amounts of light-dependent anthocyanins and hydrogen peroxide after application of relatively high amounts BABA, suggesting involvement of IBI1 in redox homeostasis. We conclude that IBI1 acts parallel or upstream of ABA in the BABA-induced priming pathway. The *ibi1-1* locus maps to a 350kb region with 114 annotated genes on the lower arm of chromosome IV. Further fine-mapping and sequencing of candidate genes are currently underway.

The CaMV35S promoter is not a constitutive promoter in transgenic sunflower plants

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Sunflower, *Helianthus annuus* L., is one of the world's major oilseed crops. Despite the importance of this plant, only a limited number of reports have described successful transformation in this species (Lucas et al. 2000, Molinier et al. 2002, Radonic et al. 2006). All these published papers were carried out with the constitutive CaMV35S promoter which is the most widely used promoter for effective transformation of both monocots and dicots. In this work, we show that in sunflower stable transformation the CaMV35S promoter doesn't have a constitutive pattern expression like in other plants and this could be the main reason why working in sunflower transformation is so unpopular. We used *Agrobacterium tumefaciens* EHA105 and EHA101 strains (Hood et al. 1993), carrying two different GUS-intron plasmids (Vancanneyt et al. 1990) as transformation vector, one for plant selection in the antibiotic kanamicyn with the *npt*II gene and the other with the *bar* gene for selection with the herbicide ammonium gluphosinate. These selection genes were regulated by the promoter and terminator *nos*, while the gus-intron was under the CaMV53S promoter and terminator. Transformation and tissue culture were performed as described previously in Radonic et al. (2006). We obtained from 5 independent T0 transformed explants a total of 18 T1 and 1 T2 transgenic plants. All T1 plants were grown in greenhouse and analysed by PCR, with 2 pairs of primers, one that amplifies a fragment from the reporter gene and the second from the selection gene. In order to investigate the expression levels of the reporter gene, RT-PCR and histochemical GUS staining assays (Jefferson et al. 1987) were performed. The use of stereoscopic lenses (20X) was necessary due to the low and specific expression of the GUS reporter gene. Remarkably the faint blue color was restricted only to abaxial face trichomes in young leaves and mainly very near to the main nerve. This expression pattern did not change when the staining procedure was longer or vacuum was used. A typical GUS staining never was detected in mesophyll or stomatal cells. Moreover, this faint expression could only be detected in 2 month old plant leaves but not in younger plants. When the vector pnos-nptII-tnos/p35S-GUSintron-t35S was also used in lettuce, the model system in the *Compositae* family, the expression pattern obtained was similar to that of other model species like tobacco or Arabidopsis, the expression was detected in different tissues and developmental stages. In 4 of our T1 plants we observed the loss of the transgene for the T2 generation, also described in our model (McCabe et al. 1999), lettuce. Our results show the need of new promoters for constitutive expression in sunflower transformation.

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Temporal and within-plant variation of induced resistance in apple seedlings

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Induction of plant resistance after initial herbivore damage is a graded process that is commonly delayed and decays after a longer period. Moreover, induction rates vary depending on plant and attacking herbivore species. In some plant species, variations in induced responses were found not only temporally but also spatially, depending on leaf age and position of leaf in relation to damaged leaf. Besides being more

pronounced, induction responses in young leaves also appeared to be more rapid and persistent compared to responses in mature leaves. This study aimed at improving the understanding of the induction process in terms of time scale and within-plant variation, using apple seedlings (*Malus domestica*) as plant model system.
We investigated the feeding preferences of chewing herbivores (*Spodoptera littoralis* caterpillars) for induced and uninduced apple seedlings. In dual choice feeding tests, six different time intervals to initial herbivore damage and three leaf positions were examined. Preference of *S. littoralis* for uninduced apple plants was first detected 1 day after initial damage and was strongest at the youngest leaf position. Induction effect persisted over several days and declined with time. Leaf position clearly affected induction of apple plant resistance.
Our findings show the temporal and within-plant variation of induced resistance in young apple plants. The observed kinetics and distribution of resistance within plants contribute to the understanding of induction processes and patterns, and support the optimal defence theory stating young tissue to be prioritized.

Possibilities on the use of resistance inducers in tobacco leaf production, results of field works in Brazil.

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Systemic acquired resistance has being studied in tobacco for a long time. But its practical use is still limited to few products, specially the ones based on salicylic acid to reduce viral infections, such as Tomato Spotted Wilt Virus. On the other hand, SAR principles fit well in an Integrated Pest Management (IPM) program for tobacco production in South America. At the same time, application of SAR inducers during the production of tobacco seedlings at floating system is practical and may protect the plants against the major tobacco diseases that are challenges during the field tobacco production. In order to better evaluate the possibilities on the use of resistance inducers in tobacco leaf production, a 2 year field trial was carried out in Rio Grande do Sul/ Brazil. Both Burley and flue-cured tobacco seedlings were treated with the following products: CleanWay every 15 days **(**100 ppm); Bionem (*P. fluorescens*) (100 mL/50 L); Hortifós e Booster every 15 days (80 mL/20 L; e 50 mL/20 L); Fitamin e Fitofós K every 15 days (350 mL/100 L; e 250 mL/100 L); Sporekill (200 mL/100 L); MaxFitus 70 at clipping (60 mL/100 L); Serenade (*B. subtillis*) every 15 days (40 mL/100 L); and Phosphilux Super 2 days before transplant (80 g/100 L). After rating for seedlings diseases, the treated tobacco plants were transplanted into a field with natural bacterial Granville wilt (*Ralstonia solanacearum*) occurrence. We observed that some resistance inducers were effective in keeping the seedlings healthy during the 65 days of the floating system. An at the field, under bacterial inoculum's pressure, products based on *P. fluorescens* and *B. subtillis* showed the best performance. The results indicate that resistance inducers are promising in triggering tobacco defenses in the seedling production, and can be effective in promoting disease resistance in the field. This approach is interesting to be implemented in an IPM program for tobacco leaf production.

Chitosan in induced resistance: more chances than limits or viceversa?

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Chitosans (CHTs), deacetylated chitin derivatives, are non-toxic and low expensive polyamines, with a variable number of amino groups available for their biological activity, which therefore depends on molecular weight (MW), viscosity and deacetylation degree (DD) of the oligomers/polymers. CHTs act as pathogen associated molecular patterns (PAMPs), able to stimulate plant own defence mechanisms, particularly against viral diseases. CHTs action mechanisms include oxidative burst, hypersensitive response (HR), callose apposition, phytoalexin and pathogenesis related (PR) protein synthesis.
We have recently shown that CHT treatments raise ABA level over three fold in bean leaves. This ABA increment mediates both callose depositions, involved in the resistance to viruses, and partial stomatal closure, which makes CHTs antitranspirant compounds as well. In spite of these interesting properties, the use of CHTs in crop protection is still hampered by some limitations, first of all its species-specific activity. This means that each crop should be treated with a specific chitosan, in term of MW range and DD. Another limitation is its solubility, though water soluble CHTs are now available. Unfortunately, chemical processing to obtain water soluble CHTs often

alters their properties, weakening their action as resistance inducers. The most common commercially available CHTs have MW over 10 kDa up to 120 kDa and over, and they must be dissolved in a solvent, usually acetic, ascorbic, or lactic acid. Though the solvent has little influence on their activity as resistance inducers, it may be determinant in impairing virus transmission by insect vectors. Solvent type can also be important when using CHTs as antitranspirant, as we have shown by measuring stomatal conductance to water vapour (G_w) and transpiration rate (E) of treated bean plants.
To screen the potential biological activity of a CHT solution on a specific plant species, we set up a rapid method based on the elicitation of callose apposition in floating leaf fragments, and correlating it with the induce resistance to tobacco necrosis virus. This method also allows testing the phytotoxicity of different CHT solutions, thus avoiding time consuming trials with numerous plants.
In conclusion, the selection of a suitable chitosan, with a homogenous composition in terms of polymer fragment length, MW range and crop-specific dilution, certainly contributes to its successful utilization as resistance inducer with other "side" beneficial effects, among them the containment of mycotoxin concentration and the enhancement of bioactive phytochemicals in plant foodstuffs.

Mycorrhizal symbiosis as a strategy for root parasitic weed control

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Parasitic weeds of the genera *Striga* and *Orobanche* spp cause severe damage to important agricultural crops worldwide (Joel *et al.*, 2007). 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 it 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 are germination stimulants for the seeds of these root parasitic plants (Bouwmeester *et al.*, 2003). In addition, it has been recently shown that strigolactones also act as host detection signals for arbuscular mycorrhizal (AM) fungi (Akiyama *et al.*, 2005). It is well known that AM fungi have a positive effect on plant fitness and on the induction of plant defense responses, conferring resistance to biotic and abiotic stresses (Pozo & Azcón-Aguilar, 2007). 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 (Lendzemo *et al.*, 2007; Sun *et al.*, 2008). Moreover, we previously showed that the tomato mutant *darkgreen* (*hp-2*^{dg}), with a reduced production of strigolactones, was less susceptible to *Orobanche aegythiaca* infection (López-Ráez *et al.*, 2008). Here we show that tomato plants colonized by the AM fungi *Glomus intraradices* and *G. mosseae* produce less strigolactones than non mycorrhizal plants and, as a consequence, induce less germination of *Orobanche ramosa* seeds. The results will be discussed in relation to the possible use of AM fungi as a suitable tool for controlling root parasitic weeds by reducing strigolactone production.

Induced resistance in pepper by Fo47 is associated to changes in gene expression

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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 (C. Alabouvette and Y. Couteaudier, 1992). In our bioassay we treated pepper plants with Fo47, then we challenged them by submerging the root in a suspension of 10^6 *Verticillum* conidia per millilitre. 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 the non pathogenic strain Fo47 and *Verticillium dahliae*. Fo47 inhibited partially the growth of the pathogen.
In order to check if gene expression associated to plant defense 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 (capsidiol), *CACHI2*, a gene that encodes a quitinase and *CAPR1*, 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. Further study is being done to check plant colonization by Fo47 and by the challenge pathogen, as well as the hormone regulation of the resistance induced by Fo47.

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Special thanks to C. Alabouvette, C. Olivain and C. Steinberg (INRA, Dijon) for providing us with the Fo47 strain.

Characterization and Cloning of Pathogen-inducible genes and promoters of *Carica papaya* to Improve resistance to *Phytophthora palmivora*

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Phytophthora species cause devastating diseases to important crop plants world-wide. The *Carica papaya*, the fifth sequenced angiosperm, has relatively few genes and is highly susceptible to the broad-host-range pathogen, *P. palmivora*. These qualities make this tropical fruit tree useful for comparative genomics of compatible *Phytophthora*-plant interactions.
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* have been 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 □-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. The significance of the genes identified here will be discussed in terms of 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

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Chlorophyll fluorescence imaging (Chl-FI) has already shown promise for in-field detection of plant diseases. Together with other imaging techniques, Chl-FI could be used in pathogen-challenged plants to obtain "disease signatures" characteristic of every pathogen (1).

We have previously used Chl-FI to monitor the infection of *Nicotiana benthamiana* plants with the Spanish and Italian strains of the *Pepper mild mottle virus* (PMMoV-S and –I, respectively). PMMoV-infected plants showed a characteristic non-photochemical quenching (NPQ) pattern in leaves that remain asymptomatic during the viral infection (2, 3). NPQ measures the energy non-used in photosynthesis and dissipated as heat. Chl-FI as well as thermal (4) and multicolour fluorescence imaging (5) allowed us to test also the different virulence of the two PMMoV strains. The set of images obtained constitutes the "stress-specific signature" of PMMoV infection in *N. benthamiana*.

In the case of *Phaseolus vulgaris* infected with either *Pseudomonas syringae* pv. *tomato* (Pst) or *P. syringae* pv. *phaseolicola* (Pph), Chl-FI could distinguish between the hypersensitive response induced by Pto and the systemic infection produced by Pph in a pre-symptomatic way (6). In addition, when plants developed a systemic infection, the NPQ pattern was a good indicator of the bacterial growth in the host plant: the lower NPQ values, the higher bacterial accumulation.

Since the imaging techniques enable the quantification of the pathogen impact on plants, they constitute a useful tool for induced resistance studies by allowing comparison of such impact in the presence and absence of the inducing agent of resistance.

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Extrinsic plant defence inducers and multitrophic interactions: the effects of β-aminobutyric acid on a hymenopterous parasitoid of aphids

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β-aminobutyric acid (BABA) is a non-protein amino acid that induces plant defences against a range of micropathogens and plant pathogenic nematodes. It has recently been demonstrated that application of BABA to plant roots can also inhibit both foliage and sap-feeding insects, such as lepidopteran larvae and aphids. We are currently examining the consequences of BABA-induced suppression of insect herbivores on the performance of higher trophic levels by using a model system that includes beans (*Vicia faba*), pea aphids (*Acyrthosiphon pisum*) and the hymenopterous aphid parasitoid *Aphidius ervi*.

Aphids exposed to BABA-treated host plants (either prior to or post parasitoid oviposition) tend to have higher mortality when parasitized, a reduced *A. ervi* emergence rate and produce smaller wasps. The reduction in parasitoid size is related to the concentration of BABA applied to the roots, and follows a pattern similar to that observed in the reduction in bodyweight expressed in the aphid host.

In choice-tests using Y-tube olfactometers, female parasitoids respond similarly to volatiles released from untreated and BABA-treated plants, both uninfested and when infested with pea aphids. Also, female *A. ervi* readily attack and oviposit in aphids reared on BABA-treated plants. Thus it appears that either *A. ervi* females cannot detect BABA or any changes it induces (in the aphids, plant volatiles or on the plant surface) or they do detect these BABA-induced changes but do not recognize them as a situation of potential harm.

NMR and mass spectrometry have identified numerous physiological changes in the host plant and aphid, as well as confirming the presence of unmetabolized BABA in both. Whether the BABA-associated inhibition of *A. ervi* is due simply to the decreased size of the host aphid, or is caused by the chemical changes that occur in plant and aphid or direct suppression by BABA present in the aphid host is being investigated.

Root-emitted volatiles analysed with Proton-TRansfer Mass-Spectrometry (PTR-MS) reveal the secret life of cabbage root fly larvae (*Delia radicum*)

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Cabbage root fly (*Delia radicum*) is a serious pest insect. The larvae feed on roots of crucifer species, thus reducing the yield and market value of rape seed, broccoli, Brussels sprouts and many other crops. Chlorfenvinphos, the insecticide that was used to control root flies, is forbidden by the EU since 2007, because of its toxicological effects on humans and the environment. Consequently there is a great need for alternative strategies to combat this pest insect, including biocontrol by natural enemies. *In vivo* monitoring of volatile organic compound (VOC) emissions of root fly infested plants may help to achieve this goal.

Previous studies have shown that entire *Brassica nigra* plants emit specific VOCs when damaged by root flies[1]. One of these compounds, dimethyldisulfide (DMDS), was shown to attract natural enemies of *D. radicum* in the field[2]. On-line PTR-MS VOC analysis showed that DMDS and dimethylsulfide (DMS) emissions from the roots indeed increased significantly12-16 hours after root fly infestation. Additionally, infested plants showed increased emission rates of mass 60 (compound m/z 59, as PTR-MS measures mass +1). The emission of mass 60 was indicative for the presence of actively feeding larvae, because it started within 4-6 h of infestation and ceased when larvae pupated or died. Artificial wounding of the roots briefly increased the emission rates of mass 60 as well. This suggests that mass 60 represents a product of glucosinolate conversion by myrosinase. The identity of the mass 60 compound is currently elucidated by GC-MS analysis. Compounds typical for leaf wounding ("green leaf volatiles") were not induced by root fly larvae or artificial root damage.

Our results show that specific VOC are emitted when crucifers are damaged by root fly larvae. This information may be used to develop equipment for monitoring infestation rates of this cryptically feeding pest. Additionally, these results are valuable for plant breeders aiming to select crops with enhanced attractiveness to natural enemies of *D. radicum*.

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Green Leafy Volatiles: Priming and Insect Attraction in a Maize Field

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Maize plants attacked by the herbivorous larvae of *Spodoptera frugiperda* emit volatile organic compounds (VOCs) that attract various parasitic wasps. Some of these volatile compounds also cause neighbouring plants to physiologically prepare themselves for subsequent herbivore attack (priming). Green Leafy Volatiles (GLVs) are volatile organic compounds that comprise C_6 aldehydes, alcohols, and their esters. Recent laboratory studies have suggested their involvement in both priming and insect attraction. These two effects were investigated under realistic field conditions in subtropical Mexico, where biologically relevant doses of a mixture of synthetic GLVs were released next to several maize plants. After 10 days of exposure to the GLVs, herbivory, predation and parasitization of *Spodoptera frugiperda* caterpillars was measured, as well as volatile emissions of maize seedlings next to the GLV-dispensers. Maize seedlings next to GLV-dispensers released significantly more sesquiterpenes than seedlings without GLV-dispensers, suggesting an important role of priming by GLVs under field conditions. Interestingly, the number of *S. frugiperda* larvae and the number of infested plants were higher in plots with GLV-dispensers than in control plots. In contrast, neither parasitization nor predation were affected by GLV-dispensers. The results suggest that the release of volatiles may affect various ecological parameters and their application in the development of novel strategies for ecologically sound pest control should be considered with caution.

Pine tree chemical defensive strategies and the evolutionary trade-off between induced and constitutive defenses

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Induced defences have been considered less expensive than constitutive preformed defences since the cost is realized only when required. But induced chemical resistance has some associate costs due to possible loses in term of fitness derived from the time required for their synthesis or re-allocation.
In pine trees, resin and phenolic compounds are the major carbon-based chemical defences. As both are linked by common carbon sources, it could be expected that they would be not maximized at the time. Furthermore, preformed defences and plastic defensive responses differ in their benefits in terms of fitness for long-lived plants. Genotypes constitutively well-defended are expected to gain little boosting their defences after damage to be protected against subsequent attacks; conversely genotypes with low constitutive defences are likely to be under the pressure for expressing effective inducible responses. The existence of these evolutionary conflicts has been many times suggested in the literature and sometimes reported for angiosperms, but rarely in conifers and not yet in pine trees. The aim of this paper was to explore the existence of this trade-off in the maritime pine (*Pinus pinaster* Ait.).
We grew pinions from 18 open pollinated mother trees from Galicia (NW Spain) in a greenhouse under controlled conditions. After two years, half of the pine seedlings were sprayed with MJ (100 mM in 0.1% Tween-20) and the remaining acting as controls (0.1 % Tween-20). We analyzed the secondary chemistry (total polyphenolics in the needles and the gravimetric resin content in the stem) and we performed an *in vivo* feeding bioassay with a generalist insect herbivore (the pine weevil *Hylobius abietis* L., a phloem-feeder herbivore) to check how the expressed defences reflected the ability to avoid the attack.
We found negative genetic correlations between the constitutive and inducible defences, measurable in the physiological defensive traits and in the effectiveness of the defensive compounds against the herbivory insect, which constitute strong experimental evidences that this genetic trade-off exists in this pine tree. We explored that these negative correlations were not spurious using a Monte Carlo iteration procedure. We confirmed strong negative genetic correlations between induced and constitutive levels of resin content ($R^2 = 0.72$), and also for the realized damage by the weevil ($R^2 = 0.71$), but the relationship observed for total polyphenolics ($R^2 = 0.48$) appeared to be spurious. The analyzed genetic entries of *P. pinaster* showed a continuous range of defensive strategies from families with reduced expression of constitutive defences which showed the ability of dramatically increase their defences after induction signals, and families with strong expression of constitutive defences which are poorly capable of increase their defences after attack.

ARBUSCULAR MYCORRHIZAL FUNGI (AMF) INDUCED INSECT RESISTANCE IN TOMATO

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AM fungal inoculation in crop plants significantly influences the morphological and physiological parameters of the host plant which in turn enhances resistance or susceptibility to the infesting insects. Keeping this in view, field and greenhouse experiments were conducted at Annamalai University, Annamalainagar, Tamil Nadu, India during 2004-2006 to study the role of four AM fungi *viz., Glomus fasciculatum, G. mosseae, Gigaspora margarita, Acaulospora laevis* on insect resistance in an already identified insect resistant (R) tomato accession, (Varushanadu Local) in comparison with a susceptible check (I-979). In the first field trial, *G. mosseae* inoculated plants of the 'R' accession, Varushanadu Local recorded less population of the fruit worm, *Helicoverpa armigera* Hubner., whereas in the second field trial, *G. fasciculatum* enhanced the resistance in the 'S' check, which recorded less larval population. In contrast, larval population of *Spodoptera litura* Fabricius was not significant among the various AMF incoulated and uninoculated plants of both the accessions. Feeding preference of both *H. armigera* and *S. litura* under confinement test was less towards the foliage of 'R' accession. Upon analysing the biochemical and biophysical factors of such induced resistance, O.D. phenol and total phenol contents of the accessions were found to influence the resistance, but with much variation between accessions and among AM fungal species. Similarly the trichome density also exerted significant influence on resistance. Further, in depth studies on the biochemical and/or molecular interactions between the host and the AM fungal species may reveal its influence on insect resistance in tomato.

The transcription factor MYC2 shapes plant defense responses in *Arabidopsis* upon *Pieris rapae* herbivory

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Plants are capable to defend themselves against a broad range of attackers. Once an attacker passes constitutive barriers, several plant hormones are produced leading to a local and systemical defense response that will counteract the attacker. It is known that the hormone jasmonic acid (JA) plays a major role in the defense 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[1] (e.g. the hormones ethylene (ET) and abscisic acid (ABA)). Transcription factors (TFs) play an important role in the regulation of the differential JA response. In *Arabidopsis thaliana* the TF MYC2 was identified as key regulator of wounding specific JA responses[2]. MYC2 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 is activated, while in *myc2* mutant plants the ERF-dependent *PDF1.2*-branch of the JA response is activated. Although this shift in transcription pattern appeared to have no direct influence on the weight gain of larvae of the small cabbage white (*Pieris rapae*), caterpillar choice tests revealed that *Pieris* larvae have a preference for plants mutated in MYC2 over the wild type. This indicates that in wild-type plants, activation of MYC2-dependent JA responses plays a role in deterring insect herbivores such as *P. rapae*, resulting in less damage to the plant.

Antimicrobial effects of extrafloral nectar (EFN)

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Extrafloral nectar (EFN) is an aqueos solution that is secreted by more than 300 plant genera. Its main function is the attraction of carnivores, which then serve an indirect defence of the plant against herbivores. Due to its high contents of sugars and amino acids, EFN appears prone to microbial infestation. We therefore investigated the antifungal defence in EFN of three obligate ant-plants (the myrmecophytes *A. cornigera*, *A. collinsii* and *A. hindsii*, plant species which are obligatorily inhabited by specialised ants) and of two EFN-secreting non-myrmecophytes (*A. farnesiana* and *Prosopis juliflora*, which only secrete EFN to attract ants from the vicinity). Myrmecophytes secrete EFN constitutively at high rates, while non-myrmecophytes secrete it in lower quantities and only in response to herbivore damage. Under field conditions, myrmecophyte EFN was free of fungi, while we detected fungi in non-myrmecophyte EFN. Extrafloral nectar of myrmecophytes contained significantly more proteins than the EFN of non-myrmecophytes, both with respect to protein amounts and numbers of different proteins. Bioassays showed that EFN of myrmecophytes efficiently inhibited the growth of yeast and of four phytopathogenic fungi, and this activity could be linked to the protein fraction of the nectar. Most proteins found in EFN of myrmecophytes could be identified as PR-proteins (> 95% of the total amount of proteins that we detected in 2D-gels and characterised by nanoLC-MS/MS). Glucanases and chitinases alone contributed more than 50% to the total protein content. EFN of non-myrmecophytes showed only activity of chitinases, and mainly acidic isoforms, while myrmecophyte EFN exhibited activity of acidic as well as basic isoforms of both chitinases and glucanases. Our study demonstrates that PR-enzymes play an important role in protecting EFN from microbial infestation and, perhaps, also in protecting the EFN-secreting plant from infections by fungi that could use nectaries as infection sites.

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

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The Enemy Release Hypothesis (ERH) is one of the mostly cited theoretical frameworks to explain how exotic species become invasive out of its natural range. This hypothesis predicts a reduction of the impact of biotic enemies on populations established in new environments, resulting in a selective advantage regarding similar indigenous species, with which they now co-occur. This hypothesis has been widely tested in different plant-animal systems and results are controversial. Some studies found exotics to be more damaged and/or more impacted in terms of fitness by herbivores than native species, whereas others found the opposite. We investigate here 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 insect herbivore, *Hylobius abietis* (Coleoptera, Curculionidae). This insect is an important forest pest in Europe that strongly hampers the regeneration of coniferous forests. Adults of this species fed on the bark and phloem of young seedlings of different coniferous species, causing stem girdling and high seedling mortality. We evaluated the effects of this insect in i) *in vitro* cafeteria experiments, ii) *in vivo* bioassays, and iii) in two naturally infected genetic trials of *P. pinaster* and *P. radiata*, jointly planted on a coniferous clear-felled area. Each trial includes 90 replicates of 31 open pollinated families of each pine species and one control of the opposed species. Contrary to the ERH predictions, one year after planting, debarked area caused by the pine weevil was significantly greater in the exotic pine in both trials. However, *in vitro* bioassays with the same material cultivated in the greenhouse 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 48 h exposing greenhouse seedlings of both species to the insect. The higher resistance of *P. pinaster* in field conditions could derive from induced resistance mechanisms preferently elicited in the native species following the insect damage. These mechanisms are unable to be expressed in cut twigs, whereas the short time of the *in vivo* bioassay may have impede the switch of the insect preferences observed at field conditions after an attack that lasted for several months. According to this hypothesis, the induction of resin in the stems (the main resistant trait in conifers) after a 48 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 defense 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.

Arbuscular mycorrhizal symbiosis enhances indirect defense of bean plants against the two-spotted spider mite *Tetranychus urticae*

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Arbuscular mycorrhizal (AM) symbiosis is suspected to "prime" plants against pathogenic microorganisms and similar effects are expected for direct and/or indirect defense mechanisms against arthropod herbivores. We studied the impact of AM symbiosis on an acarine predator-prey system consisting of the polyphagous two-spotted spider mite *Tetranychus urticae* and its specialized natural enemy the predatory mite *Phytoseiulus persimilis*. Experiments on possible direct defense against the spider mites were performed on detached leaf arenas with leaves taken from common bean plants (*Phaseolus vulgaris*) colonized or not colonized by the AM fungus *Glomus mosseae*. Juvenile survival and development, adult survival and oviposition and offspring sex ratio were assessed in order to estimate the impact of AM on the population growth parameters of *T. urticae*. To test for indirect defense *P. persimilis'* behavioral response to volatiles of mycorrhizal and non-mycorrhizal leaves of spider mite-infested *P. vulgaris* was assessed in a Y-tube olfactometer. Population growth of *T. urticae* on bean was positively affected by the presence of AM symbiosis, indicating no or a negative effect of AM on potential direct defense of bean plants against *T. urticae*. In contrast, volatiles from spider mite-infested mycorrhizal beans resulted to be more attractive to *P. persimilis* when offered alongside their non-mycorrhizal counterparts. The latter suggests that AM symbiosis enhances indirect defense of bean plants against *T. urticae*.

POSTERS

1 Evaluation of TaGSK1 in selected wheat genotypes in Iran

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In order to determine the level of TaGSK1 gene expression in 9 selected wheat genotypes an experiment was carried out in Agricultural Biotechnology institute, university of Zabol. Nine lines of wheat (*Triticum aestivum* L.) were obtained from Zabol Agricultural Research, Sistan and Baloochestan, Iran. For each wheat line 10 seed were placed in glassware containing 50 mL of solid MS-medium with 25 gL^{-1} sucrose without any growth regulators. The medium was stalinized with NaCl to make the final concentrations of 0 and 200 mol m^{-3}. Total RNA was extracted from 0.2 grams of leaves meristem using RNAsy Total RNA Isolation Kit (QIAGEN) according to manual company. First-strand cDNA was prepared from 120 ng of total RNA, using universal Oligo$(dT)_{15}$ primer and 200 units of SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA), at 42 ° C for 1 h in a 20- mL reaction volume. Each reaction was performed on 5 mL of 1 : 100 (v/v) dilution of the first-strand cDNA, synthesized as described above, in a total reaction volume of 25 mL using SYBR Green PCR Master Mix (Applied Biosystems) and 270 nM of each forward and reverse primer.
For QPCR data, Relative expression for the GOI was determined using ΔΔCt method (Livak and Schmittgen 2001). The expression of the GOI was relative to a control plant sample which was no exposed to salinity stress. Kavir line was chosen and termed the calibrator.
ΔCt(sample) = Ct (GOI sample) __ Ct (Refrence gene sample)
ΔΔCt= ΔCt(sample) __ ΔCt(calibrator)
Relative expression = $2^{-\Delta\Delta Ct}$
A different pattern of TaGSK1 transcript accumulation was found between 9 selected lines that were exposed on salinity stress and non stress conditions. The expression level for three lines including ER-salt-81-14, ER-Salt-85-12 and Mahdavi lines were at least 50% higher than other six genotypes. Also, the results of real time PCR showed that the Bam genotype has maximum level expression of TaGSK1 between 9 genotypes. Minimum expression was belonged to ER-Salt-85-17 line originated from Tehran province.
Key word: TaGSK1 gene, wheat, gene expression, QPCR

2 Study of expression of low-temperature- responsive genes for selected barley accessions in Iran

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In order to determine the level of low temperature response genes expression in ten selected barley accessions were obtained from Sistan Agricultural Research at spring of 2008. Total RNA from the shoot meristem and the root meristem in low-temperature treatment and control conditions were extracted using RNSy kit according to the manufacturer's instructions (QIA gene). The total RNA was then quantified on a spectrophotometer. For cDNA synthesis, One microgram of the DNase-treated RNA was used for first-strand cDNA synthesis using 500 ng of oligodT(12–18) , 900 ng of random primer, and 200 U of Superscript_ III according to the manufacturer's instructions.(Fermentas Revert Aid ^{Tm} first standard cDNA synthesis kit) The result of Real Time PCR, using cDNA from three low temperature response gene (blt2,blt14 and blt101), showed that there were significant difference in gene expression between three treatments and in each gene the highest percentage of gene expression belonged to accession 5 while accession 8, 9, and 10 composed one separate . In bltt14 gene, the increase in the amount of mRNA was carried out when the maximum level of freezing (4°C day / 2°C night) apply. All of these genes are shown to be transcriptional regulated and root meristem had maximum level of RNA under cold treatment.

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

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Dry pea is the second grain legume in importance world wide and the most important in Europe. One of the main constrains of the crop is the rust disease caused by *Uromyces pisi* with losses up to 100%. Breeding for resistance is the most economical and friendly method of control against this pathogen, but low resistance is available in pea so far (Barilli *et al*., 2009a). In order to validate alternative pea rust control methods, systemic acquired resistance to pea rust has been induced in pea by exogenous application of benzothiadiazole (BTH) and DL-3-amino-n-butanoic acid, β aminobutiric acid (BABA) solutions. Expression of BTH-induced SAR has been associated with transcriptional activation of gene encoding pathogenesis-related (PR) proteins promoted by endogenous accumulation of salicylic acid (SA) whereas the cellular and molecular mechanisms through which BABA exert its action are not so well reported. In addition, BABA capacity to confer protection against basidiomycetes in general, and rusts in particular, is controversy (Amzalek and Cohen, 2007).
In this work two different pea genotypes, the susceptible cv. Messire and the genotype PI347321 with partial resistance against *U. pisi* (Barilli *et al*., 2009a) were investigated in relation to fungal development following BTH (10 mm) and BABA (50 mM) treatments. The effect of treatments on β-1,3-glucanase, chitinase, phenylalanine ammonia-lyase and peroxidase activities together with analysis of excreted and total soluble and wall-bound phenolic compounds were also investigated in order to relate them to the different resistant mechanisms observed through histology.

Results show that BTH and BABA systemically impair fungal development at different stages, both prior and after mesophyll cell penetration. Fungal inhibition at early stages, before mesophyll contact and particularly during appressorium formation, was associated to the production of phenolic compounds such as scopoletin and pisatin which were found on both the excreted and the soluble fraction following both treatments. These compounds showed a similar inhibitory effect when exogenously applied *in vitro* bioassay. Penetration and post-penetration resistance was associated to the enhancement of PR-proteins such as β-1,3-glucanase, chitinase and peroxidise. However differences in the PR proteins induced were observed between BTH and BABA treatments

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4 Elicitors of *Leptosphaeria maculans* inducing resistance to blackleg in oilseed rape

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Leptosphaeria maculans is a causal agent of blackleg of oilseed rape, one of the most important diseases of oilseed rape worldwide. Control of this disease is usually achieved by fungicides and utilization of resistant cultivars. However, single gene based resistance is easily broken down owing to high evolutionary potential of this pathogen, which makes searching for alternative crop protection strategies, including induced resistance, highly desirable. Regarding to its hemibiotrophic lifestyle, induction of resistance to this pathogen is more complicated than to bitrophs and a number of inducers fail to induce resistance. We focused on elicitors produced by *L. maculans* into liquid cultivation media and preliminary chracterization of the efficient fractions. Compounds produced by *L. maculans* into various cultivation media were tested for their ability to induce defence gene expression by means of RT-qPCR and SAR by inoculation test. After removing toxins and other low molecular metabolites by dialysis, the medium was concentrated and subsequently subjected to fractionation by ionex chromatography. Separated fractions inducing *PR1* expression were digested by trypsin. Tryptic hydrolysates lost their *PR1*-inducing activity, which indicates a protein nature of the efficient elicitors.

5 Deciphering the function of *Arabidopsis* heterotrimeric G protein in innate immunity

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Heterotrimeric G proteins are well conserved signalling complex in mammals, yeast and plants that are composed of three protein subunits (α,β,γ. In *Arabidopsis*, the α(GPA1) and β (AGB1) subunits are encoded by single copy genes, while two genes encoded the γ subunit (*AGG1* and *AGG2)*. Arabidopsis heterotrimeric G protein has been described to play a relevant role in plant resistance to different necrotrophic and vascular pathogens (Llorente *et al*., 2005; Trusov *et al*., 2006), as *agb1* showed an enhanced susceptibility to these pathogens. Previous results obtained in the lab indicated that AGB1-mediated signaling was independent of JA, SA and ET signal transduction pathways. To elucidate the mechanisms controlling AGB1-mediated, we performed a comparative transcriptional analysis of wild-type plants and the *agb1* mutant. Functional classification of the proteins encoded by differentially regulated genes in *agb1*-1 after infection revealed an over-representation of proteins related with cell wall, plasma membrane and responses to abiotic and biotic stresses functions. These results further corroborates that *agb1* mutant is impaired in defence responses and suggests that *agb1* may have cell wall structure/composition modifications respect to those of wild-type plants. To further characterize AGB1-mediated resistance, a suppressor screening on a *agb1*-2 EMS mutagenized population has been performed to identify *sgb* (*s*uppresors of *G* *b*eta) mutants that restored *agb1* susceptibility to necrotrophic fungi to wild-type levels. The genetic and molecular characterization of the *sgb9*-*sgb12* mutants isolated will be presented.

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

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Fusarium oxysporum f. sp. *lycopersici* (FOL) has proved to be a protective agent in pepper against several pathogens, namely, *Verticillium dahliae*, *Phytophthora capsici* and *Botrytis cinerea* (Díaz. *et al.*, 2005). The mechanism of such protection seems to be induced resistance and ethylene signalling is needed for it (Díaz. *et al.*, 2005). Induction by FOL caused an increase in chitinase activity and cell wall phenolics as well as enhancement of expression of defense genes (Díaz *et al.*, 2005; Silvar *et al.*, 2009). The induction of resistance in plants is due to the recognition by the plant of MAMPs that trigger a response that prime or activate the resistance mechanisms. In the present study, we just started the research to try to unravel the effects of MAMPs derived from FOL. In practice, FOL could not be used for biocontrol because is a pathogen of tomato, but a MAMP derived from FOL would be agronomically acceptable.
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 upregulated by the FOL extract, both in the roots and the leaves.

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

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The grapevine is subjected to numerous forms of pathogen aggression, especially downy and powdery mildews (*Erysiphe necator* and *Plasmopara viticola*).
We sought to develop new integrated pest management strategies. To understand the impact of alternative methods like plant defence stimulators in addition to varietal resistance and biological control on the evolution of bioagressor populations, it is important to investigate the role of genetic variability and the evolutionary potential of pathogen populations subject to alternative method selection, and to decrease the risk of resistant populations.
We assessed the efficacy of two phosphonate derivatives (fosetyl-Al and PK_2, a foliar fertilizer) and the benzothiadiazole (BTH), a salicylic acid analog, on the induction of grapevine defences against various phenotypes of grape downy mildew and various genotype groups of powdery mildews in *in vitro* systems (disks of Cabernet Sauvignon vine leaves). BTH was uniformly effective against all the categories of pathogens while fosetyl was 4-fold less effective against group A powdery mildew strains than against the others. PK_2 was 6- to 7–fold less effective against powdery mildew than against downy mildew.
To confirm the stimulating effect of defences, gene level expressions were measured by RT-PCR for three dpi to assess their involvement in plant defence mechanisms. We monitored gene expressions coding for enzymes of the biosynthesis pathways of phenylpropanoïds (PAL, STS, CHS, CHI, LDOX, BAN), phytohormones (LOX, ACC, PAL) and genes coding for PR (CHIT4c, PGIP, PIN, GLU, PR1, PR10). The secondary metabolites produced were also quantified to correlate levels of gene expression and phytoalexin production.
BTH stimulated grapevine defences by triggering the overexpression of genes coding for the enzymes of the biosynthesis pathways of anthocyanins, stilbenes and PR proteins. Fosetyl had similar efficacy to that of BTH. As regards PK2, grapevine responses were limited.
The answers of grapevine defences are different after an attack of powdery mildew A or B or of downy mildew: an attack of powdery mildew A leads to stimulation of the stilbene biosynthesis pathway while downy and powdery mildews B induces the biosynthesis pathway of anthocyanins and PR proteins.
We conclude on the role pathogen diversity on the efficacy of these plant defence stimulators and also on the effective stimulation of grapevine plant defences.

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

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Plant parasitic nematodes, Arbuscular mycorrhizal fungae (AMF) and Plant Growth Promoting Rhizobacteria (PGPR) share the root system as a common environment and food source. Therefore interactions between them can have an effect on plant tolerance or resistance to plant pathogens and can be used in control of plant parasitic nematodes. We have studied the effect of the combined use of AMFs (*Glomus mosseae* or *Glomus intraradices*) and a PGPR (*Bacillus megaterium*) on nematode (*Meloidogyne incognita*) reproduction and plant growth of several commercial cultivars of pepper and tomato. A great variability in nematode reproduction and plant growth parameters was observed depending on species and plant cultivars but nematode reproduction and disease severity (estimated by gall index) was reduced and plant growth increased in all mycorrhizal plants. Plant growth reduction caused by *Meloidogyne incognita* was compensated by colonization of roots with *Glomus* spp. Nematode infestation did not affect mycorrhizal colonization of roots. We did not observe any synergic effect of the combined use of AMFs with *B. megaterium* in tomato but in pepper cv. Perico reductions in nematode reproduction were greater when *G. mosseae* and *B. megaterium* were used together than when used alone. Reductions in mycorrhizal colonization of roots in commercial tomato varieties carrying resistance genes to *Fusarium*, as suggested in literature, were only observed in one out of the five tomato cultivars tested.

9 Polyamines: putative mediators of plant resistance.

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Polyamines are plant growth regulators that have been previously associated to pathogen responses, however their role on plant resistance remains mostly unknown. Arabidopsis plants blocked in polyamine biosynthetic genes show enhanced resistance to Pst, contrastingly, plants overexpressing SAMDC and ADC2 showed hypersensitivity to the bacterium. Even though these apparent result, polyamine mutations result in overcompensation by other genes of polyamine biosynthesis rending unexpected polyamine profiles. In order to better understand polyamine roles in plant resistance, a fast analytical method for underivatized polyamines has been developed. The analysis is based on LC coupled to MS/MS by using additives to ion-pair polyamines. Ion pairing rends in a better chromatographic resolution and does not affec method accuracy.

10 Understanding the rhizobacterial-mediated induction of systemic resistance in melon

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The Cucurbitaceae is a major family for economically important species, particularly those with edible fruits. The major cultivated types include cucumber, melon, watermelon, squash and pumpkin. Melon (*Cucumis melo*) is one of the most important horticultural crops in Spain with a production of more than 1 million tons in 2006 and €337 millions in profits. Powdery mildew is a devastating disease of cucurbits especially in melon, which causes important economical losses all over the world. Fungicide applications and the use of resistant cultivars are the main means of control. Unfortunately, the limited availability of commercially acceptable resistant cultivars, the increasing problem of fungicide resistance and public concerns about the hazardous effects of chemicals on the environment, have led growers to explore environmentally friendly alternatives or complements to chemicals for the management of cucurbit powdery mildew such the use of biological control agents. Considering the ectoparasitic life style of powdery mildews, it has been often assumed that they could be efficiently targeted by mycoparasites or antibiotic-producing microorganisms; however, their use generally require a high relative humidity for optimal disease-suppressive activity, conditions fairly achieved in greenhouses but not in open field plantations. In Spain melon crops are mainly grown in open fields. For this reason, an interesting approach to overcoming this environmental restriction could be the use of rhizobacterial strains able to promote the induction of systemic resistance in the plant. In a previous work we have selected several rhizobacterial strains, two *Pseudomonas fluorescens* strains (UMAF6031 and UMAF8402), two *Bacillus subtilis* strains (UMAF6639 and UMAF6614) and one *Bacillus cereus* strain (UMAF8564), able to elicit protection in melon against cucurbit powdery mildew, achieving disease reduction values ranging from 43 to 52%. The objective of the present study is to unravel the defence mechanisms underlying the induction of systemic resistance promoted by these rhizobacteria as well as to identify the signal transduction pathways that regulate this enhanced powdery mildew resistance in melon plants. For this purpose we have selected several plant defence marker genes such as *PR-1*, *PR-5*, *LOX*, *POX*, *ETR*, *CTR*, *PAL1*, *PAL2*, and acidic and basic β-1,3-glucanase and chitinase genes, for studies of gene expression by qPCR. Furthermore, we are carrying out ISR assays against other melon pathogens such as *Pseudomonas syringae* pv. *lachrymans* and *Botrytis cinerea*, and on other cucurbit crops such as cucumber and zucchini against powdery mildew, in other to explore the range of diseases and hosts more suitable for these rhizobacteria. Moreover, we are testing these bacteria on *Arabidopsis* against powdery mildew in order to take advantage of the tools developed in this plant to study signal transduction pathways.

11 Systemic induction of bean isoflavones by a Plant Growth Promoting Rhizobacteria consortium against the leaf pathogen *Xanthomonas campestris* pv. phaseoli

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Plant growth promoting rhizobacteria are non-pathogenic bacteria able to trigger plant's defensive metabolism. In some plant species as legumes, isoflavones 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 the effect of a combination of PGPRs on growth and isoflavone contents in early stages of bean development, comparing with individual effects of each strain.

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 isoflavones 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 and the individual bacteria, one upon challenge and the second 12 days after; six days after the second inoculation, plants were pathogen challenged and one week after, disease symptoms were recorded. Based on height data from the short experiments, all strains were able to prime the plant since all decreased plant height, indicating that plants detour C metabolism to defensive metabolism compromising growth (Conrath et al, 2002 Trends Plant Sci. 7: 210–216). Despite that 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.

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12 Functional analysis of *nox1* gene of *Trichoderma harzianum* and its role in ROS production

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Several species of *Trichoderma* are biological control agents commonly used in agriculture due to their ability to antagonize other fungi. The antifungal properties of *Trichoderma* spp. have been related to the production of antibiotics and/or hydrolytic enzymes and competition for nutrients. *Trichoderma* has also the capacity to induce resistance in plants against phytopathogens and promote plant growth. Within the project Trichoest (FP5, QLRT-2001-02032), cDNA libraries were prepared using mRNA populations transcribed under mycoparasitic, nutrient stress or plant interaction conditions from *Trichoderma* strains. ESTs were generated by sequencing the cDNA clones from the 5' end.

The L03T34P05R07015 clone (EST7015) showing high identity with NADPH oxidase (NOX1), using the BlastX algorithm, was selected. It is well known that generation of a burst of reactive oxygen species (ROS) catalyzed by NOX is an important defence mechanism for both mammals and plants against invading microbes. In fungi, this kind of proteins also regulates the development of sexual fruiting bodies, hyphal growth in both mutualistic and antagonistic plant-fungal interactions, and defence. For *nox1* isolation the EST fragment was used as a probe to screen a lambda genomic DNA library (L01) of *T. harzianum* CECT 2413. A positive phage was sequenced and two oligonucleotides were designed and used to amplify the cDNA of the gene from amplified phages from the *T. harzianum* L03 cDNA library. The deduced NOX1 protein has 557 amino acids, a theoric weight of 64.3 kDa, a calculated Ip of 9.21 and six transmembrane domains. Oxygen metabolism regulation boxes could be identified at the promoter region.

nox1 expression was analysed under different conditions, such as interaction with phytopathogens or interaction with plants. High expression levels were detected at low time in the presence of *Pythium ultimum*, living plants or hypoxia, suggesting that *nox1* could be involved in defence responses and plant root colonization.

Functional analysis of *nox1* was performed by its overexpression in *T. harzianum* T34. The *nox1* implication in root colonization and plant defence induction is being evaluated using the transformant strains in *in vivo* assays.

13 Factors influencing the inhibition of aphids by β-aminobutyric acid

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β-aminobutyric acid (BABA) is a non-protein amino acid that activates or primes plant defences against a range of plant pathogens. It has also been demonstrated that application of BABA to host plants can inhibit both chewing and sap-feeding herbivorous insects, such as lepidopteran larvae and aphids. We have performed a number of trials that aim to clarify some of the biological and experimental conditions necessary for BABA-induced suppression of aphids to occur.
With whole plants, application of BABA by spraying or dipping foliage had no effect on the growth of individual aphids (in terms of bodyweight), whereas application of BABA as a root drench caused a significant reduction in the growth of a number of different aphid species (and genotypes) developing on a range of host plant families. It was not necessary for the plant to be intact for BABA-induced suppression of aphids to occur: aphid growth was also suppressed on detached leaves of *Pisum sativum* and *Medicago truncatula* where the cut end of the petiole was immersed in BABA-solution. The inhibition of aphids was isomer specific, in that BABA but not γ-aminobutyric acid (GABA) applied as a root drench or to a cut petiole caused a reduction in aphid growth. Adult aphids as well as developing nymphs were inhibited by BABA: adults maintained on BABA-treated plants exhibited decreased nymph production after 3-4 days. Although these BABA-inhibited adults displayed a loss of body weight and reduced nymph output, suggesting the quality of the host plant as a resource was reduced in some way, there was no associated increase in the production of winged (alate) progeny.
The results indicate that inhibition of aphids by BABA is a very general effect, occurring for all species and genotypes of plants and aphids so far tested. NMR and LC-MS analyses have identified a number of chemical changes in plants that occur after BABA has been applied to the roots, including the presence of unmetabolized BABA in leaf tissue and phloem. We are currently attempting to elucidate the individual or combined roles of enhanced plant defences, induced changes in plant nutritional quality and the direct physiological effects of BABA-ingestion on the resultant inhibition of aphid growth.

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

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β-aminobutyric acid (BABA) is a non-protein amino acid that activates or primes plant defences against a range of plant pathogens. Application of BABA to plant roots can also inhibit both chewing and sap-feeding herbivorous insects, such as lepidopteran larvae and aphids. Herbivorous insects may show restricted growth if the quality of their host plant is in some way compromised, and although most investigations suggest that any effects of BABA on plant pathogens are in some way linked to priming or activation of plant defences, a number of phytotoxic effects of BABA have also been observed. To give an indication of how the insect's resource is modified by BABA, we performed a series of trials to examine how various performance parameters of seven crop plant species were affected by application of BABA at concentrations known to inhibit insect performance.
Soaking seeds of *Vicia faba*, *Medicago truncatula* and *Hordeum vulgare* for 24h in BABA solution had no effect on the quantity of solution imbibed or subsequent germination of seeds. However, application of BABA as a root drench caused a reduction in shoot length and a decrease in shoot fresh weight for all plant species tested except *V. faba*. BABA application consistently resulted in an increase in shoot percent dry matter, suggesting an influence on the water-relations of the plant. The observed effects on plant growth were isomer specific, in that application of γ-aminobutyric (GABA) did not cause similar effects, indicating that any responses were not simply caused by the application of an osmoticum to plant roots.
In terms of chemical composition of the foliage, BABA caused an increase in shoot H and N concentration in both *V. faba* and *M. truncatula*. In contrast, shoot K, Fe, Ca and Mg concentrations were reduced in BABA-treated plants. This could be a contributing factor to the decrease in fresh weight and increase in percent dry matter of shoots of BABA treated plants.
The results indicate that, even without considering the role of plant defences, it is highly likely that herbivorous insects will encounter a much changed resource after

application of BABA to their host plant. We are currently utilizing NMR and mass spectrometry to investigate BABA-induced metabolomic responses in plants that may affect herbivore performance, including the modification of amino acid profiles in leaf and phloem and the induction of defence pathways and secondary metabolites associated with insect attack.

15 Functional characterization of the *PRLIP2* gene in Arabidopsis

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Over the past decades, there has been increasing evidence demonstrating that the induced resistance of plants is often associated with an enhanced capacity to mobilize stress-induced cellular defense responses – a process called priming. In a differential screening among Arabidopsis plants pretreated with the resistance-inducer β-aminobutyric acid (BABA), previously we have identified a novel pathogenesis-related (PR) gene family (*PRLIP*s) encoding lipase-like proteins. Gene expression analyses and microarray data available in the databases indicate that *PRLIP* genes may have a role in salicylic acid (SA) mediated systemic acquired resistance (SAR), as well as in BABA-induced priming.
In our present research, we have been characterizing the effects of PRLIP2 deficiency and overexpressing under normal and stress conditions. The knock-out (KO) mutant Arabidopsis (Col-0) lines S_18848 and S_34780 have a T-DNA insertion in the third exon of the *PRLIP2* gene (At5g24200), resulted in total absence of the appropriate mRNAs. Parallelly, several overexpressing Arabidopsis (Col-0) plant lines were obtained using constructs exploiting the 35S viral promoter.
During the phenotypic characterization of the KO mutant lines we found increased root growth and leaf extension compared to wild type plants. Under salt-stress conditions, the KO mutant lines still showed increased root elongation and a quicker shoot growth than wild type plants. However, BABA pretreatment diminished the difference indicating altered stress responses in the mutants.
To verify the role of *PRLIP2* gene in plant-pathogen interaction, we have examined the KO mutants in bacterial infection tests. The experiments have been performed with virulent (DC3000) and avirulent (DC3000 avrRpt2) strains of the plant pathogen bacterium *Pseudomnas syringae pv. tomato*. We found that the disease symptoms of the compatible infection with the virulent strain were more intensive on the leaves of the KO lines compared to the wild type plants. Likewise, during the incompatible interaction with the avirulent strain the symptoms of the KO lines simulated the symptoms of a compatible infection of a wild type Col-0 plant. Interestingly, the bacterial growth rates did not show significant differences in the leaves of wild-type and mutant lines. To clarify the background of this phenomenon, we analyzed the expression pattern of *PRLIP* and other stress related genes in wild-type and mutant lines.
In this poster we will summarize the results of these experiments and discuss the possible role of PRLIP2 in the priming of plant stress responses.

16 Effect of some morphological and chemical characters of corn on the resistance to the Corn Stem Borer *Sesamia cretica*

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Six corn genotypes (BOHOTH 106, CBR 2000, IPA 2052, IPA5012, SAKHA9433 and CML 329) were investigated during this research. Our results showed differences between genotypes in characters (plant length, number of leaves and leave areas). The correlation among these characters and the percentage of infestation by the Corn Stem Borer (CSB) did not appear to be significant .
Results of the field survey showed a negative correlation between the trichome density and the numbers of predator (*Coccinella undecimpunctata*). The percentage of predators was 39 % in corn plants with low, and 11 %, in plants with high density trichome genotypes, respectively. The study comprised analysis of primary and secondary metabolites present in the green parts of all corn genotypes investigated. These compounds were carbohydrates , protein, fats , fibers , lignin and coumarins.
The results of the analysis indicated differences in the amount of these compounds between genotypes. Although there were differences in the amount of the primary compounds, they did not have any influence on the infection by CSB . Lignin content in green parts of the corn genotypes ranged from 11.9 to 18.1%. This finding was correlated to the level of infestation by CSB since we found that infestation decreased as lignin concentrations increased. The coumarins were present in varying amounts

in the corn genotypes and the survival of larvae decreased with increasing coumarins concentrations.

17 The beneficial fungus *Piriformospora indica* induces fast root surface pH signaling and primes systemic alkalinization of the leaf apoplast upon powdery mildew infection

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We analysed by non-invasive electrophysiology local and systemic responses in the interaction of the plant-root-colonizing basidiomycete *Piriformospora indica* and barley roots. In the short-term (seconds, minutes), a constant flow of *P. indica* chlamydospores along primary roots altered surface pH characteristics: Whereas the root hair zone alkalizes transiently in a typical elicitor response, the elongation zone acidifies as is indicative of enhanced H^+ extrusion by plasma membrane H^+ ATPase stimulation. Eight to 10 min after treating roots with chlamydospores, apoplastic pH of leaves began to acidify in contrast to the known alkalinization response observed with various stress conditions. In the long term (days), plants with *P. indica*-colonized roots responded to inoculation with the leaf pathogenic *Blumeria graminis* f.sp. *hordei (Bgh)* with a leaf apoplastic pH increase of over 2 units while the elevation on non-colonized barley was limited to 0.8 units. The strong apoplastic pH response is reminiscent of *Bgh*-triggered pH shifts in barley leaves that are resistant to powdery mildew either due to the presence of a resistance gene or treatment with a chemical resistance inducer. It is suggested that the pH signal is transported through the xylem and leads to an activation of the plasma membrane ATPase (H^+ pump) which in turn initiates the resistance pathway in the leaves.

18 Preventive and post-infection control of *Botrytis cinerea* in tomato plants and fruits by hexanoic acid

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We have recently studied the antifungal activity of hexanoic acid on the phytopathogen *Botrytis cinerea* (Leyva *et al.*, 2008). This chemical inhibited both spore germination and mycelial growth *in vitro* in a concentration-dependent manner and stopped spore germination at a very early stage, preventing germ-tube development. Spray application of hexanoic acid at fungicidal concentrations on full developed MicroTom plants prior to *B.cinerea* inoculation reduced the necrosis diameter by approximately 15% with respect to untreated plants. Application of the same hexanoic acid concentrations on previously infected plants reduced further necrosis expansion by around 60%. A similar preventive effect was found on Ailsa Craig mature green fruits treated with hexanoic acid. The curative action of hexanoic acid was effective on fruits at different ripening stages. The results suggest that this natural compound acts as a preventive and curative fungicide for crop and postharvest aplication. Its effect in vitro was comparable with other comercial fungicides. Our results suport that hexanoic acid is a good candidate for safe antifungal treatments for the control of *B. cinerea*, which is responsible for many economic losses on fruits, vegetables and flowers.

Leyva M.O., Vicedo B., Finiti I., Flors V., del Amo G., Real M.D., García-Agustín P., González-Bosch C. (2008) Preventive and post-infection control of Botrytis cinerea in tomato plants by hexanoic acid. *Plant Pathology* 57, 1038–1046.

19 Individual Plant Growth Promoting Rhizobacteria from an effective consortium stimulate different systemic protection in *Oryza sativa* against salt stress

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Rice crops are of great importance for economic, social and environmental reasons. Spain had the highest yield in the marshes of the Rio Guadalquivir (Sevilla). This production area is in proximity to the Doñana National Park, making it a place of high ecological value, and where agricultural practices have to be careful not to cause damage to the ecosystems.
The main difficulties in the production in this area are due to fungal pathogens and soil salinity owing to penetration of the sea by over-exploitation of aquifers that decrease the water level of the river Guadalquivir. It is also known that the elicitation of the defensive system of the plant also can be initiated by certain non-pathogenic bacteria known as PGPR (Plant Growth Promoting Rhizobacteria (Cheong et al. 2002. Plant physiol. 129(2):661-677). When faced with a stress factor, the plant activates its secondary metabolism involved in defense, having ascertained that there is an overlap in the complex network of reactions involved in the process of defense against biotic and abiotic stress (van Loon et al 1998 Ann. Rev Phytopathol. 36:453-483). The plant functions as a whole with the metabolic tools available. It has been found that plants in these situations, alter the physiological state, and notable changes within the photosynthetic apparatus are detected. As a result, there is now a special interest in research aimed at developing inoculants with beneficial effects. The main purpose of these biofertilizers, is the stimulation of secondary metabolism of the plant, allowing the cultivation of species of agronomic interest in areas with a high incidence of pathogenesis, areas with high salinity or restrictions water.
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 3.5 g / 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 ΦPSII) 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" (Conrath, U. et al. 2002. Trends Plant Sci. 7: 210-216).

20 Impact of environmental factors on PAMP-induced callose in hydroponically grown Arabidopsis

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Plants respond to pathogenic microbes by depositing callose-rich papillae, which prevents further colonization by the pathogen at an early stage of infection. Recently, it was discovered that induced callose deposition is regulated by glucosinolate metabolites[1]. To further study the impact of environmental factors on this defence reaction, we studied callose deposition in hydroponically grown Arabidopsis seedlings upon challenge with two pathogen-associated molecular patterns (PAMPs), flagellin and chitosan. Seedlings exposed to high light intensity (~150 $\mu M/m^2/s$) showed significantly enhanced amounts of callose deposition than those exposed to relatively low light intensity (~75 $\mu M/m^2/s$), suggesting that light potentiates callose deposition. Conversely, increasing concentration of sucrose in the medium suppressed basal and PAMP-induced callose depositions, possibly due to a suppression of photosynthesis activity[2]. Interestingly, addition of antioxidant vitamins to the 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[3], sugar signalling[4] and ROS production[5], 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[1]. 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.

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21 Assessing antibiotic resistance of host plants (*Pisum sativum* L.) to the pea aphid, *Acyrthosiphon pisum* (Harris) in response to nitrogen fertilisation

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The performance of apterous pea aphids (*Acyrthosiphon pisum*) was assessed on host plants (*Pisum sativum*) supplied with four nitrate-N levels (0, 3, 15 and 30 mM) using various growth, development and reproductive parameters in controlled environmental conditions. The results indicated that aphids reared on plants supplied with N-free nutrient solution consistently had a longer pre-reproductive period, shorter reproductive period, shorter post-reproductive period, and shorter lifespan (longevity) than the aphids reared on N-fertilised plants. However, the differences in reproductive periods, post-reproductive periods or longevities of aphids between the 0 mM N treatment and the highest N level (30 mM) were not significant. Aphid performance, particularly in terms of adult weight, total fecundity, reproductive rate and intrinsic rate of increase (r_m) increased as N supply was increased up to 15 mM, and then declined with the highest N level (30 mM). Similar trends between treatments were confirmed by measuring the mean relative growth rate (MRGR) of aphids, although statistically only the aphid MRGR on N-deficient plants (0 mM) differed significantly from the other N treatments. The relationships between several measures of aphid performance, in particular the r_m/MRGR relationship, were examined across N treatments and were discussed.

22 Optimal defense in pine trees: constitutive and induced allocation of resin and polyphenolics in *Pinus radiata*

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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 induced strategy of optimal allocation of the two major conifer defenses, resin and polyphenolics, to two tissues with contrasting fitness value, stem and needles. We hypothesized that the upper meristematic tissues supporting primary growth should be the better defended, but definitive adult basal needles could be more defended than primary needles. We grew 72 *Pinus radiata* Don. seedlings during two years under greenhouse, half of them were treated with methyl jasmonate (80 mM MeJa in Tween 0.1%) to induce chemical defenses, leaving the other half as controls (treated only with Tween 0.1%)for studying constitutive defences. We analyzed the quantitative defensive chemistry in needles and stem, along three parts of the plants (basal, medium and upper apical part).
We found that phenolics are major defensive compounds in needles, while resin based defences appeared relevant both in stem and 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. Stem phenolics were scarce relative to leaves and did not show significant changes within the plant.
Interestingly, induced responses of pine trees to MeJa 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.

Induced rising concentrations of those compounds were not accompanied bychanges in their allocation patterns (not significant MeJa x part interaction).
Our results indicate a marked pattern of allocation of defenses along the plant and among tissues relevant for plant fitness, which constitute the first report for pine trees. Our observations escalate the Optimal Defense Theory to long lived plants, which look for optimality in their secondary chemistry allocation since the crucial very first developmental stages of their ontogeny.

23 Impact of a natural elicitor for the biological protection of a major tropical crop: the banana

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Colletotrichum musae is the main causal agent of the banana storage diseases crown-rot and anthracnose. Despite growing negative public perception, synthetically manufactured fungicides are commonly used to control these diseases. However, decreased efficacy may occur due to the build-up of pathogen resistance. Chemical fungicides are therefore under review due to the emergence of resistant strains and consumer demand. The elicitor FEN560, produced from fenugreek, has a proven effect on other plant pathologies (e.g. grapevine-powdery mildew). The aim of this project is to develop a pre-harvest treatment system against post-harvest fungal pathogens of banana using natural products.
The effect of FEN560 treatment by spraying and by watering plants and the effect of inoculating leaves with *C. musae* on the induction of defence-related enzymes (phenylalanine ammonia-lyase (PAL) and peroxidase (POX)) in the leaves were studied by UV and visible spectrophotometry. Salicylic acid was not found in banana leaves by HPLC, however other compounds not yet identified were enhanced by both FEN560 treatments.
When banana leaves were treated and then inoculated, POX and PAL activities increased. POX activity increased whether leaves were treated or inoculated, whereas PAL activity increased only in response to treatment. Thus the increase in PAL activity appears to be a treatment effect, while that in POX appears to be due to recognition of the pathogen. These increased enzyme activities suggest that the elicitor FEN560 has a preventive action for the pathosystem studied, even if the watering treatment requires priming to trigger enhanced peroxidase activity. Increased peroxidase activity is observed as plants grow, possibly correlating with higher resistance against pathogens in older banana leaves. Furthermore, the elicitor FEN560 acts as a plant growth promoter increasing leaf surface area and plant height.
Finally, these results suggest that peroxidase enzyme activity is a good molecular marker for this pathosystem and that employing FEN560 treatment as an elicitor to control post-harvest pathogens is worth further investigation.

24 Herbivore induced changes in resource partitioning

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Resistance and tolerance are key defense traits of plants. Historically resistance and tolerance responses have been viewed as alternative defense responses. While others have documented strong induced resistance in tomato, we present evidence that tolerance traits, resource sequestration and localized senescence, are also induced in this species.
Methods: Young tomato plants were subjected to three treatments: control, damage plus caterpillar regurgitant, and damage plus water. Treatments were imposed for 4 consecutive days. We conducted two experiments. In the first experiment we measured the change in chlorophyll content non-destructively during, and after, the treatment period using a chlorophyll meter. In the second experiment, we quantified resource uptake and partitioning. These plants were labeled with stable isotopes of carbon and nitrogen on day 1, harvested the plants after the damage period and quantified the distribution of isotopes in focal leaves, leaves above, leaves below, in fine roots and in storage tissues (stem and main root).
Results: Damage induced chlorophyll loss, and therefore nitrogen mobilization, in the damage treatments during the period that plants were damaged. Interestingly, chlorophyll loss was higher in the control plants after the treatment period. Both damage treatments, especially damage plus regurgitant, reduced carbon fixation and resulted in greater carbon partitioning to storage tissues. In contrast, damage increased

nitrogen uptake and showed the greatest accumulation in young tissues, perhaps contributing to induced resistance.
Conclusions: Young tomato plants respond to damage by mobilizing nitrogen and sequestering carbon in storage tissues, indicating that both tolerance traits are also induced in this species. We suggest that tolerance responses are likely to become increasingly important since insect outbreaks are predicted to increase as a consequence of climate change.

25 *Trichoderma atroviride* SC1 induces resistance against grapevine downy mildew

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Trichoderma is a cosmopolitan genus, which can colonize soils, rhizospheres and phyllospheres. Several *Trichoderma* strains are active against numerous plant pathogens, and therefore used as biocontrol agents (BCAs). The fungal strain *T. atroviride* SC1 was isolated in northern Italy in 2000 from decayed hazelnut wood. It is effective against several pathogens (i.e. *Botrytis cinerea, Armillaria mellea, Podosphaera xanthii*). *T. atroviride* acts as mycoparasite. Production of chitinase and lysis of the host cell wall of the plant pathogens are the most important steps in the mycoparasitic attack. *T. harzianum* T39 can induce systemic resistance in plants, grapevine included. This capability has not been documented for the grapevine-*T. atroviride* interaction. Our aims were to evaluate the efficacy of *T. atroviride* SC1 against grapevine downy mildew (*Plasmopara viticola*) and to characterize its mechanism of action.
Plants were grown in a greenhouse in pots. Inoculum of *P. viticola* sporangia was prepared by washing the lower side of grapevine leaves that were carrying freshly sporulating lesions with cold distilled water. Inoculation was carried out by spraying leaves with sporangia water suspension and incubating over night in darkness at 80% RH and 20°C. *T. atroviride* SC1 conidia, *T. harzianum* T39 conidia and BTH (Bion® 50WG Syngenta Crop Protection) were applied three times (three, two and one day) before inoculation on basal leaves (4-5 leaves). As control treatments, plants were sprayed with water and with copper hydroxide (Kocide 2000, Du Pont de Nemours). Incidence (percentage of symptomatic leaves) and severity (percentage of symptomatic leaf surface) were assessed at the end of incubation period. Incidence and severity on treated (local effect) and untreated leaves (systemic effect) were analysed and compared with water and copper-treated controls.
The systemic effect of *T. atroviride* on downy mildew severity was similar to *T. harzianum* T39 and BTH (significant reduction compared to water-treated control). The reduction of diseases severity on *T. harzianum* T39 and *T. atroviride* SC1 treated leaves was lower compared to BTH, which had a strong effect comparable to copper.
The two *Trichoderma* species acted similarly against the disease. Their effect is much weaker compared to the standard (copper) and, from a practical point of view, it can be regarded as a tool to reduced susceptibility of grapevine to downy mildew, rather than an alternative to fungicides. A potential use in organic viticulture could be the integration of systemic resistance inducers, as *T. atroviride* SC1 of *T. harzianum,* with alternatives to copper, in order to reach a satisfactory efficacy level.

26 Effect of exo- and endogenous influence of harpins HrpN and HrpW from *Erwinia carotovora* subsp. *atroseptica* on induced immune system of tobacco is different

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Proteins $HrpN_{Ec}$ and $HrpW_{Ec}$ from *Erwinia carotovora (Ec)* belong to the harpin family. They are acidic, glycine rich, heat stable, and capable of hypersensitive reaction (HR) induction when infiltrated into tobacco leaves. Information that application of harpins enhances induced resistance to pathogens and insects has stimulated us to check whether $HrpN_{Ec}$ and $HrpW_{Ec}$ may have a similar effect. Two ways of induction were compared: exogenous (treatment of plants with protein solutions) and endogenous (constructing plants expressing transgenes of these harpins). While studying endogenous effect it was shown that the levels of expression of several PR genes *(PR-la, PR-lb, PR-3* and *PR-5)* are higher in transgenic tobacco plants expressing $hprN_{Ec}$ It is interesting to note, that induction of HR marker gene *hinl* did not occur in transgenic lines, as was shown in plants infected with bacterial pathogen. On the other hand, expression of the $hrpW_{Ec}$ transgene in tobacco plants did not have any effect on PR genes expression. Exogenous application of harpins showed the opposite effect: $HprW_{Ec}$ induced PR genes expression but $HprN_{Ec}$

did not. This could happen due to pectate lyase homologous domain present in $HprW_{Ec}$ structure that may cause higher affinity of this protein to plant cell walls.

27 OCP3, a new regulator of the Induced Systemic Resistance

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In *Arabidopsis*, selected strains of nonpathogenic rhizobacteria from the genus *Pseudomonas* are able to trigger an Induced Systemic Resistance (ISR) that is highly effective against a broad-spectrum of pathogens. This pathogen-induced ISR functions independently of salicylic acid (SA) but requires responsiveness and intactness of the jasmonate and ethylene signal transduction pathway. Furthermore, albeit independent of the SA pathway, the activation, full expression and execution of the ISR response *in planta* requires the normal function of the NPR1 disease regulator and in particular its associated cytosolic function. In this work we describe the functional implication of OCP3 in the ISR response to different life-style pathogens such as *Pseudomonas syringae* and *Hyaloperonospora parasitica*. OCP3 was previously identified as a nuclear transcriptional regulator that is pivotal to mount an effective disease resistance to necrotrophic fungal pathogens and controls essential aspects of drought stress adaptation responses in *Arabidopsis*. In addition, we investigated a possible role of this transcriptional regulator in controlling critical aspects of the cytosolic function of NPR1 and also in the modulation of a SA/JA cross-talk in the context of plant-pathogen interactions.

28 Plant Growth Promoting Rhizobacteria trigger isoflavone metabolism in early stages of development in Glycine max var osumi

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At present, soybean is playing a crucial role both in the field of food and in pharmaceutical industry. Isoflavones have a remarkable therapeutic potential (Mateos-Aparicio et al, 2008. Nutrición Hospitalaria, 23(4): 305-312) and can be delivered either through the diet (bioactives or nutraceuticals) or as food supplements, and this has opened a new market for industry. However, due to the inducible nature of secondary metabolism, isoflavone levels change according to environmental conditions leading to inconsistent effects on health. This lack of reproducibility may be overcome by the means of elicitation (Poulev et al, 2003. Journal of Medicinal Chemistry, 46(12): 2542-2547). Biotic elicitation with Plant Growth promoting rhizobacteria (PGPR) is proposed as a useful strategy to improve biomass production and to trigger secondary metabolism at the same time, by using several mechanisms, being especially relevant those that necessarily involve plant metabolism (Ramos Solano et al, 2008. Phytopathology, 98(4): 451-457).
The aim of this work was to evaluate the effects of nine PGPR isolated from different backgrounds to alter isoflavone 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 daizin 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 (Domench et al, 2006.PLSO 290:43-50) and in tomato (unpublished data), 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, unraveling the underlying mechanisms of the systemic induction.
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29 Effects of elicitor treatment on the development of tomato and the interation of root-knot nematodes.

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Tomato, *Solanum lycopersicum* L., is an important vegetable crop, cultivated worldwide. Yield loos due to root-knot nematodes (*Meloidogyne* spp.) on tomato can reach 60% in Europe. Plants infected with *Meloidogyne* spp. have typical root galling, and some infected plants also express nutrient deficiency symptoms. Root-knot nematodes (RKN) are obligate, sedentary endoparasites of many plant species. The nematode infection elicits important changes in plant gene expression (Gheyssen and Fenoll, 2002), but the majority of upregulated genes seem to be induced through the establishment of the feeding site. These are related to metabolic pathways, cell-cycle progression, water transport and cell-wall expansion. On the other hand, several host defence genes are downregulated during attack, suggesting that the nematode actively suppresses the host defence response (Jammes *et al*., 2005).
In general, plants use constitutive and induced strategies against pathogen attacks. Plant defense involves several inducible mechanisms, including some systemic responses, which can prevent secondary infection against a broad-spectrum of microbes. In addition to pathogens, SAR is induced by exogenously applied chemicals, including 2,6-dichloroisonicotinic acid (INA), benzol (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), L-β-amino butyric acid (BABA) and hexanoic acid (Conrath et al. 2002). However, the main research has been done in plant-bacteria and plant-fungal interaction, the data about plant-nematode and the elicitor biocontrol are very scared.In previous work, our group have been checked the effect of SA-related elicitors (INA and BTH) in the tomato-*Meloidogyne javanica* interaction (Sanz-Alférez et al., 2008). More recently, we have been focusing our research in the treatment of tomato plants with BABA and hexanoic acid. In brief, we have looked for the optimal concentration of these chemicals to keep tomato growth and metabolism not impared, but to reduce the efficiency of *Meloidogyne* infection. Furthermore, we have analyzed changes in gene expression of PRs (PR1, PR3, PR5...), as well as, other genes related to the signal transduction of defense responses, such LOX, CHS, and PDF1-2. The results of this recent work will be present and discuss the possible correlation of changes in gene expression to the resistance against nematode infection. However, since the mechanisms that control the plant responses are very complex, and depend on the type of the interaction, we would need a better knowledge of this compatible interaction and the interference of exogenous chemicals.
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30 Elicitors from a biocontrol *Fusarium sambucinum* and *Pseudomonas fluorescens* protect wheat from multiple fungal pathogens

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In Non-Chernozem zone of Russia, spring wheat is attacked by multiple airborne and soilborne fungal pathogens. Of main concern are root rots agents (*F. avenaceum, F. oxysporum, F. culmorum, F. sporotrichioides*, *F. gibossum* and *Bipolaris sorokiniana*), *Stagonospora nodorum* and *Puccinia recondita* causing glume/leaf blotch and leaf rust. Chemical protection of wheat from these fungi is effective but often results in development of pathogen resistance to fungicides. Use of biogenic elicitors which protect wheat via induction of disease resistance could help avoiding appearance of resistant pathogenic forms. The goals of this research were to determine if biogenic elicitors of biocontrol microorganisms able to induce a complex resistance in spring wheat to multiple pathogens. We isolated elicitors of *F. sambucinum* strain FS-94 and *P. fluorescens* strain 197, inducing resistance in various plants to different pathogens. *P. fluorescens* synthesized protein elicitor MF-3 that was identified as peptidyl-prolyl *cis-trans* isomerase of FKBP type. FS-94 produced intracellular and extracellular protein-containing elicitors of molecular mass more 10 and 30 kDa, respectively. Both MF-3 and elicitors from FS-94 have no fungitoxicity. Our results showed intracellular elicitors of FS-94 induced systemic resistance in wheat against fungal pathogens belonging to the complex of wheat root rots. Exposure of seeds to these elicitors before inoculation of seedlings decreased incidence and severity, suppressed sporulation and

reduced infectivity of all root rot agents, except *F. avenaceum*. Extracellular elicitors of FS-94 did not induce wheat resistance to root rot. The both types of FS-94 elicitors prevented *St. nodorum* development on detached wheat leaves. No or weak lesions were observed on treated with FS-94 elicitors parts of leaves for 7 days after infection by *St. nodorum* whereas severe lesions developed on untreated leaf parts. Enzyme hydrolysis with proteinase K or short-time incubation at 100ºC nullified protective effect from *Fusarium* spp. and *St. nodorum* suggesting that elicitor activity was determined by proteins. MF-3 did not protect wheat from pathogenic Fusaria, but compensated inhibitory influence of these pathogens on root growth and was effective against *B. sorokiniana*. MF-3 induced resistance to *St. nodorum* only in combination with chitosan. Thus, intracellular elicitors of FS-94 looked most promising as inducers of complex wheat resistance to the multiple pathogens. Protection plants, resulted from seed treatment with these elicitors, from different root rots (*Fusarium* spp., *B. sorokiniana* and *Rhizoctonia* spp.) and *St. nodorum* was confirmed under field conditions by results of three-year trials. In one of the years, reduction of scab incidence (*F. graminearum*) was observed. Neither MF-3 nor FS-94 elicitors were able to induce resistance against *Puccinia recondita.*

31 Study of interactions between nematodes and host / non-host plants in a model system using plant tissue culture.

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The potato cyst nematode *Globodera rostochiensis*, soybean cyst nematode *Heterodera glycines* and root-knot nematode *Meloidogyne incognita* are economically important pests and pose considerable control problems. The use of chemical nematicides is environmentally undesirable and alternatives to nematicides are being sought. Novel control strategies may arise through an understanding of the nature of resistance and the plant responses to nematode invasion at the cellular level.
Plant-pathogen interactions begin with the recognition of a potential pathogen by the plant. Cells of plants have sensitive systems for perception of pathogen-associated molecular patterns (PAMPs) of fungi, bacteria and nematodes and respond to them with activation of defense mechanisms.
We set out to search for additional chemoperception systems of plants sensing molecular patterns characteristic for different species of nematodes. We employed an experimental system involving the cellular defensive responses of cultured tobacco cells; specifically, we measured the rapid K+ efflux, concomitant medium alkalinization and an oxidative burst when cells were treated with elicitors. The response of tobacco cell cultures to cyst and egg homogenates of *M. incognita*, *G. rostochiensis*, *H. glycines* was measured. A synthetic peptide csp15, which induced alkalinization of medium in tobacco cell suspension culture at subnanomolar concentrations (Felix and Boller, 2003), was used as a control elicitor.
Measurement of extracellular pH of the cell suspension culture of *Nicotiana tabacum* (cv. Wisconsin 38, strain #217) showed that elicitor activity of *G. rostochiensis* and *H. glycines* egg homogenates was either higher or similar to activity of csp15. However, the response to csp15 was faster (4th minute) than that of homogenates (8th minute). The tobacco cell response was stronger to homogenates of nematodes not pathogenic to tobacco (*G. rostochiensis* and *H. glycines*). The response to *M. incognita* (tobacco pathogen) homogenates was very weak in comparison with cellular response to *G. rostochiensis* and *H. glycines* homogenates.

32 Effects on the foliar nutrition in the reduction of diseases in extensive crops. Studies on field in Argentina.

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The demand of increase production of wheat, soybean and maize in Argentina have carried the extendof monoculture into new geographical areas and the increase of the production on the traditional areas;as a consequence of this, necrotrofic pathogens have increased and soil's nutrients have decreased.
With the objective of study the effect of foliar nutrition as a tool to improve the tolerance in front of diseases we made eight trails in *Glycine max*, four in *Triticum aestivum* and two in *Zea mays*,with a total of fourteen sites of evaluation and four

Agricola cycle (2005/2009). We made epidemiological andproductive studies for plots with and without foliar fertilizer application, under a DCBA design, with three repetitions, in real productivities conditions in Argentina. The products under study were Nitrofoska® Foliar PS and Fetrilon® Combi.
The results in soybean show significant reduction of *Septoria glycines* levels, whose pathogen could cause losses of seed and leafs in the crops. We cheek 50% up to 56% of severity in plots without application; and in plots with application we cheek a reduction of this disease symptom in 20% up to 50%, obtaining an increase of viable legume for knot in a 12% up to 14%. With middle yieldsof 2600 kg/ha, with a variation between 1900 kg/ha up to 3500 kg/ha, we obtain the middle yield in 3204 kg/ha, with the highest production in 4000 kg/ha, that represent improvements of the 23%. That improveshave been obtained also because of the reduction of bacterial infections (*Pseudomonas syringae pav. glycines y Xanthomonas axonopodis pavar. Glcyines*) because the crops with application show leafs with better foliar structure that decrease the injury`s susceptibility.
In the works on maize the study object was the definition of the best phenological moment for thenutrient`s application in leaf. We compare treatments in vegetative period (8 leafs totally expanded, V8) and in flowering period (R1). The epidemiological following showed the symptom reduction in the diseases caused by *Exserohilum spp.* and *Helminthosporium spp.*. Severities of 15% decrease up to 8% and severities of 40% decrease up to 17%. Inboth cases the better results in final sanity were in the treatments in flowering period with significant differences with the plots without application and the plots with applications in V8. This tendency was reflected in the yieldtoo because the application in V8 allowed improvements in 5% up to 8%, and the application in flowering period allowed improvements in 7% up to 12%, that mean an increase of up to 1000 kg/ha.
In wheat crop the study objective were foliar marks caused by *Pyrenophora tritici-repentis* y *Septoria tritici*. We made a comparison of nutrients application in Zadosk 2.3-3.1 and Zadosk 4.0-4.5 scale.These trails were made in geographical areas where the agriculture is in expansion, in whatwheat is in fields with high humidity all the treatments show a less level of necrosis in leafs, with statistical significance comparing with the controlwithout nutrients and with 60% of symptoms reduction. The productivity resulted positive in all the evaluation`s sites showing that in dry cycles were more effective the nutrition in Zadosk 2.3-3.1 with improvements in 9% up to 43%. In wet years were better the treatments in Zadosk 4.0-4.5 showing yieldimprovements up to the 46% and comparing with the controlwithout treatment show significant differences with the 60% decrease in severity, in wheat with French origin.
The obtained results confirm the importance of foliar nutrition with micronutrients as a complementary tool in disease's management strategies to obtain a sustainable agro system.

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

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The induction of systemic acquired resistance (SAR) by applying acibenzolar-S-methyl (ASM) is a recommended practice in the integrated control programs of several plant pathogens. With the aim of evaluating the effects of this resistance elicitor on the spread of Tomato Yellow Leaf Curl Disease (TYLCD) in protected tomato crops, during 2007/2008 three trials were carried out in commercial greenhouses located in one of the major horticultural districts of Sardinia (Italy). In keeping with an integrated approach to crop protection, the efficacy of ASM was evaluated along with the use of non-woven row covers (NWRC) during the first weeks of the cropping period, tactics adopted in recent years by a considerable number of growers on the Island.
In 2007, in a crop planted in August and covered for two weeks with non-woven fabric, two different modes of applications of ASM were compared: a) four post-planting treatments (dose 15 mg/L a.i.) at one-week intervals (between week 2 and 5 postplanting - ASM1) and b) one pre-planting (10 mg/L a.i.) and three post-planting treatments (15 mg/L a.i.) at two-week intervals (between week 2 and 6 postplanting - ASM2). During this experiment TYLCD infection progressed very slowly, to such an extent that two months after planting disease incidence was below 10% in all treatments. Under these epidemic conditions, only treatment ASM2 was found to have any significant effect in reducing the spread of the disease compared to the untreated control. In both greenhouse experiments carried out in 2008, ASM was tested alone and in combination with NWRC for a three-week period. In each experiment ASM was applied five times, one before planting (10 mg/L a.i.) and four after planting (30 mg/L a.i.), at one-week intervals in the first trial (between weeks 1-4 of the cropping period) and at two-week intervals in the second trial (between weeks 1-7 of the cropping period). In the former case, ASM significantly reduced the percentage of plants infected by TYLCD compared to the untreated control. On the other hand, in the experiment where ASM was applied at two-week intervals, the high disease pressure observed at the beginning of the growing period lead to a rapid spread of infection

throughout the crop, with significant differences between covered and uncovered plots, but not between ASM treated and untreated plants.
These results 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 progression through the crop. During the tests conducted, ASM achieved the most interesting results when moderate infection progression was treated with frequent applications, starting from the nursery and covering an adequate period of time. The use of NWRC can contribute to limiting disease pressure, a condition regarded as favourable to the deployment of effective plant defence responses. However, the definition of an integrated control strategy based on the combination of these two tactics requires accurate adjustment of ASM applications not yet available for tomato crop protection.

34 Soilborne fungi alter the composition of tomato leaf compounds

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Over the past years modifications in the composition of plant compounds due to plant-pathogen interactions have received increasing attention. In the present paper the influence of soilborne pathogenic fungi and biocontrol agents on the antioxidant activity and the content of phenols in tomato leaves was studied. In addition, the question of a possible specific change of the chemical composition induced by different fungi was examined. In our study the tomato varieties "Kremser Perle" and "Moneymaker" were inoculated with the soilborne pathogenic fungi *Fusarium oxysporum f.sp. lycopersici*, *Fusarium oxysporum f.sp. radicis-lycopersici* and *Thielaviopsis basicola*, and with bioprotective strains of *Fusarium oxysporum* and *Trichoderma atroviride*. Mock-inoculated plants were used as control treatments. Methanol extracts of tomato leaves were prepared at three development stages and the antioxidant activity was determined with two assays (DPPH and FRAP). The content of total phenolics was determined according to the Folin-Ciocalteu method, the content of flavonoids by the use of an adapted Dowd method. Compounds of the epidermal cuticula were analysed using GC-FID and GC/MS. In leaves of mock-inoculated plants a stronger antioxidant activity was found than in plants inoculated with soilborne fungi. The leaf total phenolic content and the flavonoid content showed similar results. By applying the discriminant analysis to the changes in the epicuticular waxes, an allocation of individual samples to the respective inoculum was partly possible indicating species-specific effects of soil borne fungi on the composition of leaves.

35 Seasonal Population Fluctuation of *Etiella zinckenella* (Lepidoptera: Pyralidae) on Ten Soybean Cultivars in Tehran, Iran

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The limabean pod borer, *Etiella zinckenella* (Lepidoptera: Pyralidae) is one of the most destructive insects on pods and seeds of soybean. Seasonal population fluctuation and sampling program of *E. zinckenella* were evaluated on ten soybean (*Glycine max* (L.) Merril) cultivars including Zane, Dpx, Sari, Gorgan3, Williams, L17, 032, 033, Clark and Sahar in Tehran region, during 2008. Spatial distribution pattern of *E. zinckenella* was determined on these cultivars of soybean using regression models (Taylor's power law and Iwao's patchiness). Mean seasonal infestation among various cultivars were compared using one-way ANOVA. If significant differences were detected, multiple comparisons were made using the Student-Newman-Keuls procedure ($P<0.01$). Our results indicated that population density was highest on Sari (11.38±3.46) on the 11th of September in Tehran, Iran. The mean seasonal infestation was highest on Sari (4.51±0.88) and lowest on Gorgan3 (0.04±0.03) cultivars. There was significant differences between the mean seasonal infestation among the various cultivars (df=9, 109, F=6.806, P=0.000), thus it is possible to transfer the desirable genes from Gorgan3 (Resistance cultivar) to other cultivars in the future programs. Spatial distribution pattern of this pest wasdetermined to be random on 032 cultivar and aggregated on the other cultivars. Determining the sampling program and mean seasonal infestation of limabean pod borer can be used in integrated management of this important pest in soybean fields.

36 Role of a trichodiene synthasa gene of *Trichoderma brevicompactum* as elicitor of antimicrobial activities, and defence and development plant responses

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Species of the common soil fungus *Trichoderma* are used in the biological control of plant pathogenic fungi. The most frequently suggested *Trichoderma* mechanisms of biocontrol include mycoparasitism, competition for nutrients and antibiosis, as well as plant growth promotion and plant defense signaling activation. It is known that *Trichoderma* produces a wide variety of medically and agriculturally important secondary metabolites. Three kinds of compounds are mainly produced by different species of this genus: peptaibols, polyketides and terpenes, some of them with antifungal activity. The mycotoxin trichothecenes are fungal sesquiterpenes that have important consequences for both human and animal health but other fungal sesquiterpenes such as abscisic acid and gibberellins are plant hormones. There are works about their chemical structure and biosynthetic pathways that have been elucidated after isolation and detection of intermediate compounds. However, little information exists about genes involved in their biosynthetic pathways. The trichothecenes constitute a family of more than 60 sesquiterpenoid metabolites produced by several fungal genera, including *Trichoderma*, *Fusarium*, *Botrytis* or *Trichothecium*. We wanted to explore the trichotheces in *Trichoderma* by the isolation and characterization of genes related to its biosynthetic pathway. We cloned the *tri5* trichothece gene of *Trichoderma brevicompactum* IBT40841 (*Tbtri5*) by screening of a genomic DNA library with a probe obtained by PCR from *Fusarium sporotrichioides*. Nucleotide sequencing of the genomic DNA and cDNA revealed that the *Tbtri5* gene has an open reading frame comprising 1231 nucleotides, and contains one intron of 25 bp. This gene is homologous to those of trichothecenes from fungi and it is present in a single copy in the genome of *T. brevicompactum*. Real-time PCR analysis showed that *Tbtri5* mRNA is induced *in vitro* by different carbon sources and oxidant agents. Its functional analysis was carried out by *T. brevicompactum* overexpression using an *Agrobacterium* mediated transformation. Transformant strains showed enhanced levels of *Tbtri5* transcripts and antimicrobial activity in comparison with wild type strain.

37 Induced resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat – disease severity and production of the pathogen infectious structures.

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Effect of several possible, as well as, known resistance inducers of synthetic and biological nature was tested in powdery mildew (*Blumeria graminis* f.sp. tritici)-wheat pathosystem on leaf segments. Segments were prepared from the leaves of inducer-treated susceptible wheat cultivar Kanzler (susceptible standard) and placed on water benzimidazole agar in plastic boxes. A mixture of genotypes of the pathogen virulent to the genes of resistance *Pm*2, 6 and *Pm*4b was used as inoculum. Leaf segments were inoculated in a settle tower. Four replications were made. Induced resistance was determined according to disease severity that was evaluated after 12 days by a nine-point assessment scale. Treatment of plants by inducer was made 7 days before inoculation by powdery mildew. Following inducers were used: water solution of benzothiadiazole (BTH), salicylic acid (SA); glycine betaine /GB) and water extracts from oak bark (OB), *Reynoutria sacchalinesis* (RS), curcuma (CU), ginger (GI). Untreated Kanzler was used as a control (CO). Influence of inducers BTH and GB on the retardation of powdery mildew infection process was studied in more detail. To gain knowledge about mechanism participating on resistance induced by GB, changes in both *B. graminis* spore germination and development of infectious structures were investigated. In the development of infection process following structures were monitored: non-germinating spores, primary germ tubes formation (PGT), production of appressoria (AP) 21 h post inoculation; and non-germinating spores, PGT, AP, secondary hypha (SH), and elongated secondary hyphae (ESH) formation 48 hrs post inoculation. Microscope observation of 200 infections units was done in each time point.

All inducers under study lowered powdery mildew severity comparing to untreated CO. The lowest disease severity was found after treatment by BTH. In GI- and SA-treated plants disease severity did not extend 50 % of CO. More than 50 % of powdery mildew severity of CO showed plants treated by OB and GB. Application of CU decreased disease severity to 45 % in comparison with 70% in CO. Effectiveness of inducers in powdery mildew suppression was clearly demonstrated.
Microscopy observation revealed the effect of BTH and GB on the formation of secondary and elongated secondary hyphae, which was clear 48 hrs after inoculation. These inducers probably did not have impact on germination of conidia till 21 hrs after inoculation, which indicates that glycine betaine acts as an inducer of resistance against *B. graminis* in wheat.
To conclude, compounds of biological origin as oak bark, curcuma, ginger and *R. sacchalinesis,* and glycine betaine are promising inducers of wheat resistance against *B. graminis*, which could merit more attention.

38 Effect of Hexanoic acid against hemibiotrophs in tomato plants

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Tomato plants treated with hexanoic acid displayed enhanced resistance to *Pseudomonas syringae* DC3000 strain. A decrease in disease symptoms and in bacteria growth in leaves was observed 72hours after inoculation. Expression of marker genes involved in plant defence was analyzed along the experiment. After analysis of ACCOx and ASR1, as markers of ET and ABA signaling pathways respectively, no significant differences were observed between infected plants with or without hexanoic treatment. However, a higher and earlier expression of PR1, a SA pathway marker gene, was observed in treated and infected plants with respect to untreated and infected plants. We also observed a significant increase of jasmonic acid marker gene expression at earlier times (up to 48hpi). Analysis of hormone content in leave's samples showed a increase of OPDA, a JA precursor, in treated and infected plants, but no changes of JA and of SA were observed. In order to confirmed implication of OPDA or another oxylipins, upstream and downstream OPDA gene expression was analysed.

39 Systemic Induction of Plant Defense Responses by Synthetic Ultrashort Cationic Lipopeptides

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A new family of synthetic membranolytic ultrashort lipopeptides composed of only four amino acids linked to fatty acids was recently demonstrated to have antimicrobial activity against plant pathogenic fungi and bacteria at micromolar concentrations (Makovitzki et al., 2007). The same peptides were now tested for their ability to induce systemic resistance and defense responses in plants. We found that two peptides (C16-KK*K*K and C16-KL*L*K) can induce medium alkalinization (MA) of tobacco suspension-cultured cells and expression of defense related genes in cucumber and Arabidopsis seedlings. Moreover these compounds can prime systemic induction of antimicrobial compounds in cucumber leaves and systemic protection against the phytopathogenic fungus *Botrytis cinerea* B05 and bacteria *Pseudomonas syringae* pv *lachrimans* (Psl) and *Pseudomonas syringae* pv *tomato* DC3000.
Thus, short lipopeptides are a new category of compounds of potentially high utility in the induction of systemic resistance in plants

40 Methyl Jasmonate fails to induce resistance of *Pinus pinaster* seedlings against *Fusarium oxysporum* and *F. circinatum*

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Fusarium oxysporum is one of the causal agents of damping-of in forest nurseries. Moreover, *Fusarium circinatum* is known to cause mortality in nurseries and pitch canker in mature pine plantations. We conducted two experiments to determinate if Methyl Jasmonate (MeJa) induces defense responses in *Pinus pinaster* seedlings against *F. oxysporum* and *F. circinatum*

Two experiments were carried out with seeds of *Pinus pinaster* collected from one tree from *Noroeste litoral* (Cangas de Morrazo, Pontevedra, Spain). Seeds were surface sterilized with 30% H_2O_2 for 30 minutes, and sown on sterilized ground in individual 250 ml containers. A randomized complete block design was followed (n=20). In the first experiment plants were treated with 0, 0.1, 0.5, 1, 5, 10 mM MeJa in water, containing 0.1% (v/v) Tween 20. The solution was applied along the stem with a small brush and repeated once within 5 min to ensure an even coating. One week after treatments, half plants were inoculated with *F. oxysporum*, pipeting 5 ml onto the stem (10^6 spores/ml). In the second experiment, seedlings were grown being submitted to 8 combined treatments of N, P and K, during 3 months. After fertilization treatments, MeJa was sprayed at concentrations of 0 and 25 mM, and next month, seedlings were inoculated by contact mycelium of *F. circinatum* with a wound in the stem.

Plants showed chlorosis and wilting of needles in non-inoculated controls because of Tween 20. In inoculated plants, higher concentrations of MeJa treatments generally resulted in a significant increase of mortality by *F. oxysporum*. Differences in mortality caused by *F. circinatum* in fertilized or in unfertilized plants were not significant. MeJa did not induce resistance of *P. pinaster* seedlings against the two pathogens, probably because plants at this early stage (2- to 14-weeks old) were not able to assimilate enough molecules of MeJa necessary to trigger the resistance mechanisms. In addition, at doses above the threshold of 5 mM, MeJa caused mortality to *P. pinaster* plants, and the surfactant Tween 20 was found to cause toxicity, weakening the plants.

41 Induction of systemic resistance of tomato to root-knot nematode by biogenic elicitors

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Root-knot nematodes *(Meloidogyne* spp. Chitwood) are soil-borne roundworms that parasitize the root systems of a wide variety of crops, including cultivated tomato *(Lycopersicon esculentum* Mill). In present investigation the mechanisms of induced tomato plant resistance to root-knot nematode were studied. Biogenic elicitors - chitosan, arachidonic acid (AA), salicylic acid (SA), jasmonic acid (JA) for the modulation of immune plant responses were used. The systemic effects of biogenic elicitors on *M. incognita* infection were measured on tomato cultivars Karlson treatment with elicitors or water (control) (10 plants/treatment group). Forty-eight hr after chemical treatment, the sand surrounding the roots of each plant was injected with ~ 3,000 second-stage juvenile (J2) nematodes. Plants were maintained in a greenhouse (~24-27°C*;* 16:8 L:D photoperiod) long enough for the nematodes to complete their life cycle (6.5 wk), and nematode establishment and reproduction was compared by measuring the number of egg masses produced per plant.

Biogenic elicitors were shown to stimulate the growth and weight of tomato plants infected by *Meloidogyne incognita.* The treatment of tomato seeds with AA (10^{-6} - 10^{-7} M) or chitosan significantly suppressed the number of galls and eggs produced and increased duration of nematode development. SA($7x\ 10^{-7}$- $7x10^{-8}$ M) and JA (10^{-7}M -10^{-4}M) had no protective effect against *M. incognita,* but the compositions of SA ($7x10^{-8}$M) + AA (10^{-10} M) and JA (10^{-7} M) + AA (10^{-10} M) significantly induced resistance of tomato plants to nematode.

Also the changes of biochemical mechanisms of induced resistance in plants were steadied. It is shown that invasion of nematodes into plants leads to changes :

- in composition and content of free sterols vitally important for pathogenes;
- activity of chitinase and β-1,3-glucanase , which can destroy cell walls of parasite and in so doing to influence on nematodes vitality, and also to produce oligomers with immunoregulated properties ;
- activity peroxidase , taking part in hypersensitive death of plant cells and strengthening of plant cell walls, which restrict the nematode development;
- activity of phenylalanine ammonium lyase - key enzyme of phenyl-propanol cycle in plants
- activity of lipoxygenase, which leads to the formation of signal molecules, taking part in transduction process and intensifying the elicitors activity of pathogen.

The data obtained suggest that the mechanisms natural and induced by biogenic elicitors tomato resistance to the nematode have the same origin.

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Keywords: Resistance, Maritime pine, Methyl Jasmonate, *Fusarium oxysporum*, *Fusarium circinatum.*

42 Efficiency of some bacteria in biological control of cotton leaf worms

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Cotton leaf worms (*Spodoptera littoralis*) are the most effective pest affecting the productivity of the economically-important cotton crop in Egypt. The cotton leaf worms are traditionally controlled by the environmentally hazardous pesticides. To reduce these hazards, the recent tool, the biological control, using bacterial enemies, was adopted. The efficiency of thirty bacterial strains against leaf cotton worms (*Spodoptera littoralis*), was investigated. These bacterial strains were originally isolated from dead spiny bollworms (*Earias insulana*). Leaf cotton worms fed for four days on castor bean (*Ricinus communis*) leaves soaked for 30 minutes in suspensions of four-days old bacterial cultures. The percentages of mortality for bacterial species ranged between 10-60 %, however, five strains were highly offensive, being killed about 40-60 % of leaf cotton worms. Preliminary characterization of the bacterial strains revealed that all strains were gram positive rod-shaped with variable morphology. Five strains were identified as spore-forming *Bacillus* species, while the rest of the strains were non-spore-forming rods, which remain to be identified. These bacterial strains are good candidates for biological control of the destructive cotton pests (leaf worms and spiny or pink bollworms), an effect which improves the productivity of the cotton crop in Egypt.