Identification of the VvFLS2 grapevine flagellin receptor by a functional genomics strategy

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Contents

Session 1. Biotic and abiotic factors affecting induced resistance

Abstract: Expression of induced resistance under field conditions will be influenced by both abiotic and biotic factors. Resistance can be induced by mutualistic symbioses such as mycorrhizal colonisation, as well as by pathogen and insect attack. In turn, inducing resistance can affect mutualistic symbioses, and lead to trade-offs in defence responses. In addition, the abiotic environment can also influence the expression of induced resistance. The plant's attempts to orchestrate its responses to these multifarious environmental factors probably account for the incomplete disease or pest control achieved using resistance inducers. Providing our expectations of induced resistance are realistic, it will have an increasingly important role to play in crop protection in the future.

Sub-Session 1.1. Molecular mechanisms of induced defense

Abstract: *Arabidopsis* heterotrimeric G protein is required for an efficient defence response against different types of pathogens, including necrotrophic fungi such as *Plectosphaerella cucumerina* (*PcBMM*). Null mutants in the Gβ subunit (*agb1*) exhibit enhanced susceptibility to this pathogen, while mutants in the Gα subunit (*gpa1*) display a resistance phenotype. We have demonstrated that the *agg1 agg2* double mutant is as susceptible as *agb1* plants to *PcBMM*. To elucidate the molecular mechanism underlying the heterotrimeric G protein-mediated resistance, we performed a comparative transcriptomic analysis of *agb1-1* mutant and wild-type plants upon inoculation with *P. cucumerina*. This analysis, together with metabolomic studies, demonstrated that G protein-mediated resistance was independent from the salicylic acid (SA)-, jasmonic acid (JA)-, ethylene (ET)-, abscisic acid (ABA)- or tryptophan-derived metabolites defensive signalling, as these pathways required for resistance to necrotrophs were not impaired in *agb1-1* and *agg1 agg2* plants. Notably, in *agb1-1* transcriptome we found an overrepresentation of misregulated genes related with cell wall functions, which showed a similar expression pattern in *agg1 agg2* plants. Biochemical analyses of cell walls from G-protein mutants and wild-type plants revealed that xylose content was lower in *agb1* and *agg1 agg2* mutants that in wild-type

Sub-Session 1.2. Priming for enhanced defense

Sub-Session 1.3. Herbivore-induced defenses

recognition to immune responses, we additionally show that besides standard PTI responses, the application of *At*Peps induces the accumulation of JA, a master hormone in herbivore defense.

Abstract: *Rhododendron simsii* hybrid or pot azalea is the most important flowering pot plant in Belgium, with an annual production of approximately 35 million plants. Problems caused by broad mites (*Polyphagotarsonemus latus*) have become increasingly important in recent years. Since European legislation forces growers to implement Integrated Pest Management (IPM) in their cultivation process from 2014 onwards, several new strategies are being devised for the control of broad mites in pot azalea. One potential new strategy consists of eliciting induced resistance in pot azalea by applying the natural hormone jasmonic acid. Here we present the first results in which we test the role of methyl jasmonate (MeJA) as an elicitor inducing the jasmonic acid pathway in *Rhododendron simsii* hybrid.

Abstract: Plants have evolved general and specific defense mechanisms to protect themselves from diverse enemies, including herbivores and pathogens. To maintain fitness in the presence of enemies, plant defense mechanisms are aimed at inducing systemic resistance: in response to the attack of pathogens or herbivores, plants initiate extensive changes in gene expression to activate "systemic acquired resistance" against pathogens and "indirect defense" against herbivores. Recent work revealed that leaf infestation by whiteflies, stimulated systemic defenses against both an airborne pathogen and a soil-borne pathogen, which was confirmed by the detection of the systemic expression of pathogenesis-related genes in response to salicylic acid and jasmonic acid-signaling pathway activation. Further investigation revealed that plants use self-protection mechanisms against subsequent herbivore attacks by recruiting beneficial microorganisms called plant growth-promoting rhizobacteria/fungi, which are capable of reducing whitefly populations. Our results provide new evidence that plant-mediated aboveground to belowground communication and *vice versa* are more common than expected.

Sub-Session 1.4. Induced defense by beneficials

Abstract: *Bacillus thuringiensis*, which is a well-known and effective bio-insecticide, has attracted considerable attention as a potential biological control agent for the suppression of plant diseases. Treatment of tomato roots with the "filter-sterilized cell-free filtrate" (CF) of *B. thuringiensis* systemically suppresses bacterial wilt caused by *Ralstonia solanacearum* through systemic activation of the plant defense system. Comparative analysis of the expression of the *Pathogenesis-Related* 1 [*PR-1(P6)*] gene, a marker for induced resistance to pathogens, in various tissues of tomato plants treated with the CF on their roots suggests that the *B.*

thuringiensis-induced defense system is activated in the leaf, stem, and main root tissues, but not in the lateral root tissues. At the same time, the growth of *R. solanacearum* was significantly suppressed in the CF-treated main root tissue but not in the CF-treated lateral root tissues. This distinct activation of the defense reaction and suppression of *R. solanacearum* was reflected in differences in the transcriptional profiles in the main and lateral tissues in response to the CF. In the CF-treated main root tissue, but not CF-treated lateral root tissue, the expression of several salicylic acid (SA)-responsive defense-related genes was specifically induced, whereas jasmonic acid (JA)-related genes were specifically down-regulated in response to the CF. On the other hand, the induction of genes encoding ethylene (ET)-related proteins occurred equally in both main and lateral root tissues. Taken together, the co-activation of SA-dependent signaling pathway with ET-dependent signaling pathway and suppression of JA-dependent signaling pathway may play keys roles in *B. thuringiensis*-induced resistance to *R. solanacearum* in tomato plants.

Abstract: Enhancement of plant resistance is a promising alternative to chemical fungicides for controlling crop diseases, but its efficacy is affected by several factors. In order to further optimize the use of resistance inducers against downy mildew of grapevine, we characterized the molecular mechanisms activated by the beneficial microorganisms *Trichoderma harzianum* T39 (T39). T39 treatment directly activated the complex microbial recognition machinery and improved the phosphorylation of proteins of the signal transduction cascade. Transcriptomic and proteomic analyses revealed that T39-induced resistance partially inhibited disease-related processes and specifically activated defence responses after pathogen inoculation. However, the efficacy of T39-induced resistance was significantly affected by abiotic stresses and grapevine genotype. Combined heat and drought stresses reduced the efficacy of T39 and attenuated the modulation of defence-related genes. Moreover, expression analysis of biomarkers of T39-induced resistance highlighted complex differences among grapevine cultivars. These results demonstrated that abiotic stresses and cultivar responsiveness should be carefully considered to maximize the effect of resistance inducers.

Abstract: When applied in open fields, the efficacy of biological control agents may be strongly affected by environmental factors such as temperature and water availability. The protocooperation between *Bacillus amyloliquefaciens* S499 and plants has been chosen as a model to investigate how temperature and water regime may modulate the beneficial interaction between the two bionts. On this purpose, the effect of temperature on the ability of S499 to induce systemic resistance and to produce surfactin *in planta* has been investigated in this work.

Abstract: The necrotrophic pathogen *Alternaria alternata* f. sp. *lycopersici* (AAL) causes dark-brown canker formation on stems and tissue necrosis on leaves of tomato plants. Filamentous fungi belonging to the genus *Trichoderma* have long been recognized as agents for the biocontrol of plant disease and promote induced systemic resistance (ISR). In this study we demonstrate the

ISR effect of one isolate of *T. harzianum* against disease caused by AAL in plants of tomato cv. Micro-Tom. Disease symptoms were evaluated fourteen days after inoculation with *T. harzianum* and seven days following challenge inoculation with AAL. It was found that *T. harzianum* reduced approximately by 26.4% the disease symptoms in tomato plants compared to control plants not inoculated with *T. harzianum*. Plant genes involved in regulation of ethylene (ET) biosynthesis and response were reprogrammed by the pathogen and *T. harzianum*. The results obtained in this work suggest that *T. harzianum* is capable to increase resistance by decreasing the role of ET in susceptibility during AAL colonization of tomato leaves by attenuating ET biosynthesis and responses.

Posters

Abstract: Plant defence inducers (PDIs) constitute a class of bioactive compounds with an emerging potential to be integrated in plant protection strategies. Plant extracts such as grape marc extract (GME) could elicit plant defence responses in different species. Foliar infiltration of GME to tomato and *Arabidopsis* leaves resulted in the appearance of macroscopic symptoms and autofluorescent compounds.

Abstract: Glucosyltransferases UGT74F1 and UGT74F2 are the major responsible for the synthesis of the salicylic acid (SA) conjugates glucosyl salicylate (SAG) and salicylic glucosyl ester (SGE). The relation of these two genes and plant defense against *Pseudomonas syringae* DC3000 (*Pst*) is investigated in this work. The mutants *ugt74f1* and *ugt74f2*, altered in the corresponding glucosyltransferases were affected in their basal resistance against *Pst*. *ugt74f1* showed enhanced susceptibility visible as more pronounced disease symptoms, while *ugt74f2* showed enhanced resistance against the same pathogen. Both mutants have to some extent, altered levels of SAG and SGE compared to wild type plants, however, in response to the infection, *ugt74f2* accumulated higher levels of free SA at 24 hpi compared to wild type plants while *ugt74f1* accumulated lower SA levels. These results suggest that *UGT74F2* negatively influences the accumulation of free SA, hence leading to an increased susceptibility due to reduced SA levels.

Abstract: In response to a single acting biotic or abiotic stress, the overproduction of reactive oxygen species (ROS) takes place, which can be effectively balanced by ROS-scavenging enzymes and/or non-enzymatic antioxidants. The extent of ROS-scavenging enzyme engagement acts as a marker of stressed-plant protective potential against oxidative damage. Little is known on the involvement of ROS-scavenging enzymes in plant defence against a combination of biotic and abiotic stresses. Under field conditions, maize plants including transgenic lepidopteran insect-resistant maize (*Zea mays* L.) expressing *cry1Ab* gene from the bacterium *Bacillus thuringiensis* var. *kurstaki* (*Bt*) are frequently exposed to numerous biotic and abiotic stresses, such as non-target pests and soil-water deficit.

Abstract: In this paper we describe our strategy to unravel the connection between plant defenses and plant resistance to pests. With tomato (*Solanum lycopersicum*) as the reference plant, our ambition is to screen different plant defense stimulators (SPD) and measure (i) the resulting protection level towards *Phytophthora parasitica*, *Botrytis cinerea* and *Oidium neolycopersici* (ii) the genes involved in the corresponding defense mechanisms (iii) the proteins and secondary metabolites which could be effector of resistance. We already show that BABA induces a strong resistance to *P. parasitica* (100% protection, no symptoms). Conversely, SA does not protect tomato to *P. parasitica* infection even though some classical defense genes are highly up-regulated in the same manner after BABA and SA treatments. Preliminary results of RNA-Seq evidence that the expression of more than 1000 genes is modified by BABA treatment. The possible involvement of unreported functions is discussed.

Abstract: Nitric oxide (NO) is an important biological messenger in living organisms, involved in numerous physiological processes. Particularly, it has been shown that NO performs a vital role in the adaptive response of plants facing pathogens. During the past several years, our team demonstrated that NO is rapidly produced and acts as a signaling compound in plant cells challenged by elicitors of plant defense. One major challenge is to determine how the correct specific response is evoked, despite shared use of the NO signal and, in some cases, its downstream second messengers. Here, we will present and discuss two mechanisms by which NO exerts its effects.

First, part of NO biological functions could arise as a direct consequence of chemical reactions between NO and/or its derivatives with target proteins. Using a proteomic approach, we identified several proteins modified post-translationally by NO though S-nitrosylation in response to cryptogein, an elicitor of the tobacco defense responses. This include CDC48 CDC48 (cell division cycle 48), a member of the AAA+ ATPase (ATPase associated with various cellular activities) family acting as a chaperone-like protein. Functional analysis showed that NO inhibits CDC48 ATPase activity and triggers local conformational changes within the protein. Second, NO also controls physiological processes by modifying gene transcription. In this context, we have identified a network of genes regulated by NO endogenously produced in response to oligogalacturonides (OGs) in *Arabidopsis thaliana* leaf tissues using whole genome transcript analyses. Our analysis has pointed out the different cellular processes modulated by NO at the transcriptional level involved in plant resistance.

Abstract: Antimicrobial peptides (AMP) are small molecules found in all organisms showing antibacterial as well as antifungal activity against a wide range of pest species. Plant AMPs constitute important component of plant´s defence machinery, and besides their direct antimicrobial effect, some of them function also as endogenous elicitors of plant defence mechanisms. We focused on non-plantsynthetic AMPs as possible resistance inducers in oilseed rape (*Brassica napus*).The animal peptide chrysophsin and an insect peptide anoplin induced expression of defence genes regulated by salicylic acid and ethyleneand reactive oxygen species accumulation in treated cotyledons. Both peptides displayed fungistatic effect to *Leptosphaeria maculans in vitro*, however, only anoplin retarded symptom development in inoculated cotyledons.

Abstract: *Bacillus amyloliquefaciens* strains are tested for their ability to improve stress handling by plants. The bacteria colonize the roots and stimulate root growth. Certain *Bacillus* strains also provide disease suppression dependent on jasmonic acid (JA) signaling. The ability of these *Bacillus* strains to protect oilseed rape (*Brassica napus*) to insect pests often depending on JA was therefore investigated. *Bacillus* protection to the generalist African cotton leaf worm (*Spodoptera littoralis*) was found but not to the *Brassica* specialist Diamond back moth (*Plutella xylostella*). The application mode affected the protection, though. The chemical resistance inducers BABA and BION did not provide protection to the insects. The *Bacillus* strains show potential for plant protection and further tests aim to define the requirements for efficient protection.

Abstract: The PhytoBio INTERREG IV project involves several research teams (University of Lille, University of Champagne Ardenne, University of Gent, University of Liège, and University of Littoral Côte d'Opale) that aims at developing and studying biosurfactants of bacterial origin. Phytobio focuses on cyclic nonribosomal lipopeptides (LPs), which could be used as resistance inducers or biopesticides against plant pathogens and could be considered as an potential alternative to chemical pesticides. Our contribution to PhytoBio aims at characterizing mycosubtilin, surfactin and fengycin, three bacterial LPs produced by the rhizobacterium *Bacillus subtilis*, for their activity against two fungal pathogens of wheat responsible for two of the most important foliar diseases, *Blumeria graminis* f.sp. *tritici (Bgt),*an obligate parasitic fungus responsible for powdery mildew and *Mycosphaerella graminicola (Mg),* an hemibiotrophic fungus causing septoria leaf blotch. An *in vitro* study showed that surfactin had no direct effect on germination and on fungal growth of *Mg*. However fengycin slightly decreased the conidial germination rate and impaired *Mg* growth, whereas mycosubtilin completely inhibited both *Mg* conidial germination and fungal growth. On the other hand, no direct antifungal effect of LPs was observed *in vitro* on *Bgt* conidia germination. Moreover, a preventive treatment *in planta* with surfactin and mycosubtilin resulted at least in 41% and 44% protection levels against *Bgt.* RT-qPCR was then performed in order to study the ability of surfactin and mycosubtilin to induce the expression of defence-related genes in either non-infectious conditions or in the presence of *Bgt*. For instance, in non-infectious conditions, both surfactine and mycosubtilin induced the expression of a peroxydase encoding gene (*POX381).* However, in infectious conditions, only surfactin induced the expression of this gene in response to *Bgt*. Depending on the targeted pathogen and on the type of LP, these molecules could exhibit direct antifungal effect and/or defence induction, the later occurring through either elicitation or potentialisation.

Abstract: Some strains of plant growth-promoting rhizobacteria (PGPR) elicit induced systemic resistance (ISR). Previously, volatile organic compounds (VOCs), including acetoin and 2,3-butanediol, emitted from PGPR were identified as bacterial determinants of ISR. We screened for ISR using a microtiter plate and I-plate bioassay in which seedlings were challenged with the pathogen *Pseudomonas syringae* pv. *maculicola* ES4326 in the presence of bacterial volatiles. To investigate the induction of ISR signaling by the VOCs emitted from the reference strain, GB03, and test strain, *Paenibacillus polymyxa* E681 which showed strong capacity on ISR

and plant growth promotion under *in vitro* and field conditions. To identify plant signaling pathways involved, we screened *Arabidopsis* plants transformed with *PR1*::GUS and *PDF1.2*::GUS, indicators of salicylic acid and ethylene signaling, respectively. GB03 elicited ISR via ethylene-dependent signaling, as indicated by elevated *PDF1.2* expression in exposed seedlings, while E681 increased SA-dependent signaling, as indicated by elevated *PR1* expression suggestive that E681 and GB03 strains activate different signaling transduction pathways. The efficacy of induction was also strain-specific, with stronger protection against *P. syringae* in plants exposed to VOCs released from E681 versus plants exposed to GB03. Among more than thirty low molecular weight identified volatile compounds, including methanethiol, isoprene, and an acetic acid-butyl ester, hexadecane, a C16 hydrocarbon was found to be released exclusively from only strain E681 and can prime transcriptional levels of *PR1* defense gene. These results provide the first evidence for the existence of a novel E681 signal molecule that can serve as a bacterial determinant in ISR against *P. syringae*.

Abstract: Plants modulate defence signalling networks in response to different biotic stresses. The present study evaluated the effect of a phloem-sucking aphid on plant defence mechanisms during subsequent pathogen attacks on leaves and rhizosphere bacteria on roots. Plants were pretreated with aphids and/or the chemical trigger benzothiadiazol (BTH) seven days before being challenged with two pathogenic bacteria, *Xanthomonas axonopodis* pv. vesicatoria (*Xav*) as a compatible pathogen and *X. axonopodis* pv. glycines (*Xag*) as an incompatible (nonhost) pathogen. Disease severity was noticeably lower in aphid- and BTH + aphid-treated plants than in controls. Although treatment with BTH or aphids alone did not affect the hypersensitive response (HR) against Xag 8ra, the combination treatment had a synergistic effect on the HR. The aphid population was reduced by BTH pretreatment and by combination treatment with BTH and bacterial pathogens in a synergistic manner. Analysis of the expression of the defence-related genes *Capsicum annum pathogenesis-related gene 9* (*CaPR9*), *chitinase 2* (*CaCHI2*), *SAR8.2* and *Iipoxygenase1* (*CaLOX1*) revealed that aphid infestation resulted in the priming of the systemic defence responses against compatible and incompatible pathogens. Conversely, pre-challenge with the compatible pathogen *Xav* on pepper leaves significantly reduced aphid numbers. Aphid infestation increased the population of the beneficial *Bacillus subtilis* GB03 but reduced that of the pathogenic *Ralstonia solanacearum* SL1931. The expression of defence-related genes in the root and leaf after aphid feeding indicated that the aboveground aphid infestation elicited salicylic acid and jasmonic acid signalling throughout the whole plant. The findings of this study show that aphid feeding elicits plant resistance responses and attracts beneficial bacterial populations to help the plant cope with subsequent pathogen attacks.

Abstract: Downy mildew is one of the most destructive grapevine diseases and resistance inducers could be promising alternatives to chemical fungicides. We previously showed that *Trichoderma harzianum* T39 induced resistance against downy mildew in grapevine. Our aims were to select novel beneficial microorganisms with improved ability to induce resistance and to finely characterize their mechanism of action against downy mildew. In particular, the strain *T. atroviride* SC1 (SC1) induced local and systemic resistance in grapevine. It showed also direct activity against *Plasmopara viticola* sporangia *in vitro*. SC1 could be a valuable biocontrol agent against downy mildew. However the identification of key cellular processes is required to further develop robust resistance inducers.

induce systemic resistance. The inducers acibenzolar-S-methyl (BTH), *Trichoderma harzianum* T39 (Trichodex) and nutrient broth (NB) and isolates of the yeast *Rhodotorula glutinis* and the bacterium *Pseudomonas fluorescens* were applied to the root zones of strawberry plants as drenches. Strawberry powdery mildew was affected by the frequency of irrigation; the limited irrigation regime (once every 2 days) restricted the development of disease. All of the drench treatments induced resistance under all three irrigation regimes, but disease suppression was less pronounced when irrigation was less frequent (watering once a day or once every 2 days, as opposed to twice a day). The growth of the powdery mildew-infected plants was affected by the frequency of irrigation and was promoted by the inducers. In some cases, the growth-promotion effect of the inducer was nullified by the water shortage.

Abstract: We previously reported that the composite application of the plant growth-promoting fungi (PGPF) with the arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* (Gm) to the soil during planting improved plant growth and suppressed plant diseases caused by pathogens with different lifestyles. To elucidate the mechanisms by which the PGPF and Gm suppress symptom development of leaf pathogens in cucumber plants, we carried out a molecular study. Leaf and/or root samples were taken from plants grown in soil medium amended with the PGPF and/or Gm at sowing, at different periods after challenge inoculation with the pathogens. Results indicated an elevated expression of defense-related genes. We hypothesize the involvement of multiple defense mechanisms leading to ISR in cucumber against the leaf pathogen *Colletotrichum orbiculare* and the Cucumber mosaic virus-Y (CMV-Y).

Abstract: Previous work on basal and induced resistance by different pathogens like *B. cinerea* and *P. syringae* point to an important role of OPDA in plant defense responses. To check the role that OPDA has in defense of tomato plant against pathogens, we silenced the 12-oxophytodienoate reductase3 (*opr3*) in *Lycopersicon esculentum* cv Monney Maker plants. The analysis of gene expression as well as the hormone levels in *opr3* mutants infected with *B. cinerea* demonstrates that the mutant undergoes severe alterations in *OPR3* gene expression. Furthermore, the susceptibility of these mutants to *B.cinerea* was accompanied by a decrease in the levels of OPDA and in an almost abolished production of JA-Ile.

Abstract: We observed that protection against grape downy mildew achieved by resistance inducers was higher in the adult leaf than in the younger, not fully expanded leaf. Using sulfated laminarin as inducer, this difference of efficacy could be correlated to stronger defense reactions (*i. e.* H_2O_2, defense gene expression) in adult leaf as compared to the younger one. These findings should be taken into account in disease control strategies involving induced resistance.

Abstract: Herbivore-infested plants often release a variety of volatile organic compounds (VOCs). Here, we studied the effects of feeding *Plutella xylostella* (L.) (0, 3, 9, 20 pest larvae within 0-4 h and 4-8 h infestation, respectively) on the emission of sulfur-containing VOCs in *Arabidopsis thaliana* (L.) Heynh Col-0 (*A.t.* Col-0) by headspace solid-phase micro-extraction coupled to gas chromatography - mass spectrometry (HS-SPME-GC/MS). The analytical results showed that the relative emission of sulfur-containing metabolites increased significantly in *Arabidopsis* plants subjected to *P. xylostella* infestation according to the density and residence duration of pest larvae on shoot organs. The main compound from infested plants was dimethyl disulfide. We suggest that the correlations between the stress level (density and time infestation) and the sulfides observed in this study provide a means to understand the changes of VOCs profile of plant under chewer infestation.

Abstract: Rice leaves were treated with photodynamic dyes or 2-mercaptopyridine N-oxide, which are light-dependent sources of reactive oxygen species (ROS). As a result, disease severity was reduced on blast-challenged upper leaves. The effect was diminished by one-day darkening of treated plants or by co-treatments with antioxidants. The light-activated compounds increased superoxide production and ROS-dependent fungitoxicity in diffusates of systemically protected leaves. It is suggested that light-driven formation of ROS in treated leaves induced systemic acquired resistance, which also was realized involving ROS.

Session 2. Moving from model plants to crop plants

Abstract: Lacking specialized immune cells and exposed to a large amount of various attackers, plants are heavily relying on a highly adaptive inducible defence system. Their immune responses occur both locally with the induction of pattern- and/or effector-triggered immunity, as well as systemically with the induction of systemic acquired resistance and induced systemic resistance. These defence responses are pivotally regulated by molecular and chemical signals. In the past, various signals have been deciphered in dicot plants such as *Arabidopsis*, whereas the knowledge in crop plants is more elusive.

Sub-Session 2.1. Chemical induction of defense

Abstract: The use of vitamins including vitamin B1, B2 and K3 for the induction of systemic acquired resistance (SAR) to protect crops against plant pathogens has been evaluated previously. The use of vitamins is beneficial because it is cost effective and safe for the environment. We previously reported the use of folate precursors including *ortho*-aminobenzoic acid for the induction of SAR against a soft-rot pathogen in tobacco. In the present study, *para*-aminobenzoic acid (PABA, also referred to as vitamin Bx) was selected owing to its effect on the induction of SAR against *Xanthomonas axonopodis* pv. vesicatoria in pepper plants through greenhouse

screening. Dipping of pepper seedlings in a 1 mM PABA solution in field trials induced SAR against artificially infiltrated *X. axonopodis* pv. vesicatoria and naturally-occurring *Cucumber mosaic virus* (CMV). Expression of the *Capsicum annuum pathogenesis-related 4* gene was primed in response to pathogen infection as assessed by quantitative real-time PCR. The accumulation of CMV RNA was reduced in PABA-treated pepper plants at 40 and 105 days post treatment. Unexpectedly, fruit yield was increased in PABA-treated plants, indicating that PABA-mediated SAR successfully protected pepper plants from infection by bacterial and viral pathogens without significant fitness allocation costs. The present study is the first to demonstrate the effective elicitation of SAR by a folate precursor under field conditions.

Abstract: Induction of resistance to plant viruses causing localized infections has been widely used to study HR and SAR mechanisms. However, in Nature true virus diseases are produced by viruses able to systemize in the plant and SAR is scarcely effective against them. Thus, a more successful strategy relays in the induction of resistance against both the virus and its vector. In this work, using the pathosystem bean common mosaic virus (BCMV)-*Phaseolus vulgaris* we made attempts of inducing resistance separately to both the pathogen and the aphid vector *Myzus persicae*, with the aim of dissecting the two resistance levels inducible with the most used chemical elicitors. Results showed that BTH and chitosan are able to reduce the infection degree in BCMV mechanically inoculated plants, however not preventing the infection. On the other hand, chitosan and 2-isobutyric acid (IBA), applied as root-drench, could reduce aphid population by half. Therefore, combining the two effects and using chitosan, partially effective against both the virus and the vector, it could be possible to raise an acceptable resistance level in the field, where BCMV is actively spread by aphids. To verify this hypothesis, experimental transmission with viruliferous aphids in chitosan and IBA treated plants are now in progress.

Abstract: Nowadays, one of the most severe pathogens affecting citrus plants is canker disease. As there are no effective treatments, damage to fruit yield and quality causes huge economic loss every year. Current control measures are based mainly on SAR inducers, or on massive applications of copper which can be toxic for plants and the environment. In recent years we have been working on strategies based on natural compounds and we have recently described the effect of carboxylic acids as an inducer of resistance on citrus plants against fungus.

Application of 1 mM of hexanoic acid in irrigation water or spray on 1-year-old citrus plants clearly reduced lesions and the number of bacteria on leaves. We observed that some of the most important mechanisms involved in induced resistance were affected by hexanoic application. The results obtained show enhanced callose deposition in treated and infected plants and an induction of JA marker genes, such as *AOS*. The mechanism of action seems to act by enhancing the JA pathway and promoting callose deposition, as previously described in tomato (Vicedo *et al.*, 2009) and in citrus against *A. alternata* (Llorens *et al.*, 2013). These findings highlight the effectiveness of the JA-mediated pathway in alleviating outbreaks of citrus cankers, which provides valuable clues to design alternative preventive approaches to combat this devastating disease.

Abstract only

Sub-Session 2.2. Microbe- and plant-derived elicitation of defense

Abstract: We investigated the possibility to induce systemic acquired resistance (SAR) in barley in order to generate a monocotyledonous SAR pathosystem to test possible protection of cereals via SAR/priming. Infection of the first leaf of 4-week-old barley plants with either *P. syringae* pathovar *japonica* or *Xanthomonas translucens* significantly enhanced resistance in the systemic tissue against *X. translucens*. *P. syringae* growth was restricted in the infected leaf and caused numerous brown spots reminiscent of HR lesions. *X. translucens* seemed virulent, causing spreading lesions, severe yellowing and eventually death. We have performed microarray analyses of the local infected and systemic tissue to investigate which genes are induced and/or repressed during systemic resistance induction in barley. Results reveal that salicylic acid is dispensable, while interplay of other plant hormones is required for the induced resistance response.

Abstract: Since classical disease assays are labour- and time-consuming, and even more so in tripartite interactions, the discovery of general markers for induced systemic resistance (ISR) can greatly facilitate the search for novel or more efficient biocontrol organisms. We have reported that application of *Trichoderma hamatum* T382 to *Arabidopsis thaliana* roots resulted in ISR against leaf infection by the necrotrophic pathogen *Botrytis cinerea* and performed a genome-wide analysis of ISR-related leaf gene expression, both before and after *B. cinerea* infection. In addition, we recently completed a similar microarray analysis with tomato replacing *A. thaliana* in the tripartite interaction. Based on the comparison of the transcriptomic analyses in both plants, a series of orthologous genes up-regulated in both tripartite systems was selected. In a next step, we evaluated which of these genes could be considered as general markers for *Trichoderma*-induced ISR. Our analysis revealed 4 promising ISR marker genes, which we used to develop a screening system for fast and effective visual detection of ISR-inducing *Trichoderma* spp., based on pMarker-GUS lines in *A. thaliana*. The potential of the pMarker-GUS lines as a screening tool for identification of ISR-inducing *Trichoderma* strains was demonstrated by the clear correlation between the percentage of disease reduction and the degree of staining of the pMarker-GUS lines. Most importantly, the ISR-inducing *Trichoderma* strains identified by the pMarker-GUS lines in *A. thaliana* also showed ISR against *B. cinerea* in tomato, thus proving the validity of our screening approach.

Abstract: The surfactin lipopeptide secreted by *Bacillus* as well as rhamnolipids and the n-alkylated benzylamine derivative produced by *Pseudomonas* species, retain some plant resistance eliciting effect and belong to a specific class of MAMPs with biosurfactant activity. In this study, we first observed that the three compounds retain a similar potential to induce resistance in *Arabidopsis* and tomato plants against *Botrytis cinerea*. However, by looking at some molecular mechanisms involved in the plant response, it appeared that perception of the three elicitors might lead to different defense signatures.

Abstract: Biochar is the solid product of biomass pyrolysis. Recently, biochar has been found to induce systemic resistance to foliar pathogens of tomato, sweet pepper and strawberry. In this study, we investigated the induced resistance pathway that is mediated by biochar in the tomato – *Botrytis cinerea* pathosystem. Greenhouse waste biochar that was produced at 350 and 450°C (GHW-350, GHW-450) and included in the potting mix at a rate of 1 or 3% (w/w) induced resistance to *B. cinerea* in tomato. Despite the fact that different biochars were used and the fact that the effects of particular biochar varied in a cultivar-dependent manner, disease severity was significantly reduced in all of the tested tomato cultivars. The addition of GHW-450 to the potting mix induced resistance to *B. cinerea* in an ethylene-insensitive mutant, *Never ripe*, and in a transgenic tomato line that cannot accumulate salicylic acid (NahG), but not in a jasmonic acid-deficient mutant, *def1*. The addition of biochar to the potting mix induced the expression of *PI2*, *TomLoxA*, *TomLoxC*, *TomLoxD*, *Pti4*, *Pti5*, *GluB*, *CHI9*, *SAMT*, *ACO1*, and *PR1a* at least in one of the genotypes. Similarly, following *B. cinerea* infection, the transcription levels of the rest the genes mentioned, except for *TomLoxA* and *TomLoxC*, increased. However, in the case of the *def1* mutant, the presence of biochar in the potting mix hardly affected the expression of these genes. In conclusion, the results of our quantitative disease assay and the observed induction of gene expression strongly suggest that the jasmonic acid-signaling pathway plays an important role in the biochar-mediated resistance to *B. cinerea* observed in tomato.

Posters

Abstract: Catalase inhibitor aminotriazole or superoxide dismutase inhibitor diethyl dithiocarbamate applied to rice leaves reduced the disease severity on those blast-inoculated upper leaves which emerged later. Both treatments induced superoxide production in inoculated leaves. It is suggested that the local boost of reactive oxygen levels may have subsequent effects leading to systemic acquired resistance. The resistance may also be realized through oxidative burst in the protected leaves, which were primed by the treatments.

Abstract: In agriculture specific genotypes are commonly used as rootstocks in commercial orchards due to their good rooting properties and response to abiotic stresses and pathogens. It is known that different citrus rootstocks can affect the fitness of *Tetranychus urticae* in the leaves of the same variety. The resistant rootstock (sour orange) increases the levels of the OPDA and JA in the undamaged distal leaves in infested plants and also in grafted varieties. These results suggest that a mobile signal is transmitted from rootstock to upper parts and it could be a precursor of the oxylipin pathway.

Abstract: The *Arabidopsis At*Peps emerge as new paradigms for plant endogenous danger peptides. Upon detection by the two PRRs (pattern recognition receptors) PEPR1 and PEPR2 (Pep-receptor 1 and 2) they trigger a PTI (pattern-triggered immunity)-like response. *At*Peps

reside in the C-terminus of the small precursor proteins PROPEPs and are thought to be released under yet unknown circumstances. Recently, based on sequence and functional similarity, a PROPEP-like precursor protein as well as *At*Pep-like peptide have been identified in *Zea mays* plants, designated *Zm*PROPEP1 and *Zm*Pep1, respectively. However, no data was presented on whether maize could detect *At*Peps and vice versa. Our work shows that maize plants are insensitive to *At*Peps and likewise, *Arabidopsis* plants do not recognize *Zm*Pep1. Moreover, *At*Pep1 detection seems mainly limited to the family of Brassicaceae. Intriguingly, based on sequence analyses we identified *At*Pep-like peptides and corresponding PROPEP orthologs in most of the currently sequenced genomes of higher plants. Thus, we will now investigate, if these peptides are functional orthologs of *At*Peps. Therewith we might show that the danger signaling system consisting of *At*Pep-like peptides, PROPEPs and PEPRs is widespread within the kingdom of higher plants, but that it diverged from a common ancestor towards an incompatibility between distantly related *At*Pep-like peptides.

Abstract: 1,4-benzoxazin-3-ones (BXs) are the major secondary defence metabolites in many grasses. Over the past years, their role in resistance against insect pests has been studied extensively in maize. However, little is known about how the spatial and temporal variability of BX accumulation in maize leaves determines herbivore feeding patterns. To answer this question, we investigated how BXs shape the foraging behaviour of two insect herbivores, which differ in their degree of host-plant adaptation, on maize plants at different growth stages. Whereas *Spodoptera frugiperda* was able to tolerate high concentrations of BXs and preferentially fed on younger leaves irrespective of plant age, the generalist *Spodoptera littoralis* was more susceptible to BXs and showed a clear shift in its feeding behaviour on older plants that contain less BXs. The spatial feeding patterns of *S. littoralis* were well correlated with the distribution of BXs within the plant. Our results suggest that the defensive investment by the plant determines the feeding patterns of the two herbivores, thereby providing an example of phytochemically driven herbivore behaviour.

Abstract: In tomato leaves, production of H_2O_2 took place after treatment with licorice leaf extract (P1) and two fractions of P1, F6 (mainly flavonoids) and F4 (terpenoids and sterols), similarly to former findings in cucumber. Up to 15-fold increase of ethylene concentration compared to the control was measured in cucumber leaf samples treated with P1 and F6. Moreover, chlorophyll fluorescence (F_v/F_m) measurements in non-infected cucumber leaf discs treated with P1 and F6 showed higher F_v/F_m values than the control over 14 to 21 days (21 and 35°C, respectively). Infected cumber plants prophylactically treated with the fractions F6 and F4 as well as the raw extract also exhibited a better function of the photosynthetic apparatus than non-treated plants. The results give evidence for induced resistance by ethylene signal transduction and for general stress reduction as modes of action of *G. glabra* extract.

Abstract: Pattern recognition receptors (PRRs) emerge as important components of plant disease resistance in plants. PRRs mediate detection of potential pathogens *via* the perception of

pathogen associated molecular patterns (PAMPs). A well-studied PRR is the *Arabidopsis* leucine-rich repeat receptor kinase (LRR-RK) FLS2 that recognizes bacterial flagellin. Until now, functional FLS2 orthologues have been characterized in *Nicotiana benthamiana*, rice and tomato. We have shown that flg22 triggers a battery of defense responses and induces partial resistance to a necrotrophic fungus *Botrytis cinerea*. The putative grapevine FLS2 orthologue, designated as VvFLS2, was identified by an *in silico* approach and its functionality was demonstrated by complementation of the *Arabidopsis fls2c* null mutant. In parallel, a silencing approach in grapevine was developed to obtain stable transgenic lines silenced in *VvFLS2*.

Abstract: *Trichoderma* species are cosmopolitan soil fungi, with some strains having potential as biocontrol organisms, since they can induce systemic resistance (ISR) in plants against leaf-borne pathogens. The mechanisms of *Trichoderma*-mediated ISR have mainly been investigated in *Arabidopsis*, while few studies have attempted to characterize the ISR in the *Trichoderma*-tomato system. In this study, we tested the ISR-inducing ability of *Trichoderma hamatum* T382 against the necrotrophic fungal pathogen *Botrytis cinerea* in tomato. For this purpose, a lab-scale hydroponics set-up was first optimized, which allowed us to obtain a robust ISR phenotype, as indicated by a significant reduction of necrotic lesion diameters on the leaves. In a next step we tested several tomato mutants and transgenic lines to investigate the role of the major plant hormones in the ISR in our tripartite system. We focused on jasmonic acid (JA; mutants *spr1* and *spr2*), salicylic acid (SA; transgenic line *nahG*) and ethylene (ET; ethylene insensitive mutant *Nr*). Our results indicate that the ISR triggered by *T. hamatum* T382 in tomato is JA biosynthesis-dependent, while a role for SA and ET signaling was not found.

Session 3. How to predict success in the field from success in the lab?

No abstract

Sub-Session 3.1. In forest trees

Abstract: Previous greenhouse studies have shown that jasmonate-inducible responses provide resistance against the pine weevil, a phloem chewer causing large conifer seedling mortalities at field. Here we explore whether elicitation of the immune system in conifer seedlings before planting could be efficient in protecting seedlings at real field conditions. We performed a large collaborative field experiment with four conifer species widely planted in North (Norway spruce and Scots pine) and South Europe (Maritime pine and Radiata pine) in Sweden and Spain, respectively. Seedlings were treated with four methyl jasmonate (MJ) concentrations before planting in an area naturally infested by the weevil. Chemical defences, seedling growth and weevil damage were studied during two growing periods after planting. In general, MJ treated plants showed increased quantitative defences, and were the less attacked, less wounded, less girdled and less killed. Effects were mostly dose dependent, although some interactive effects with species were observed. Even when MJ treatment had a growth cost, height of the treated

plants did not differ too much from that of untreated plants after two years, due to the benefits of inducing defences when the intensity of damage was high. Elicitation of MJ-inducible defences in seedlings at the nursery could be an efficient and environmentally friendly way of preventing the extensive mortalities caused by the weevil after planting. An extended analysis of this experiment is currently under review elsewhere (Zas *et al*., submitted).

Abstract: Forest nurseries in New Zealand produce over 50 million radiata pine seedlings (*Pinus radiata* D Don) annually including seedlings, rooted cuttings and micro-propagated plantlets, and this involves a considerable level of chemical intervention to manage the fungal diseases affecting production. A global trend towards reduced fungicide input has raised interest in alternatives to traditional chemical control strategies and the NZ forest industry is supporting research to investigate the use of defence elicitors and beneficial endophytes to enhance plant resistance and to suppress pathogens. The introduction of ecologically sound biocontrol methods would reduce the dependence on high-risk chemicals for disease management. In this paper we present a brief review on the use of *Trichoderma* spp., and the chemical elicitor methyl jasmonate in the glasshouse, the nursery and in the forest plantation.

Sub-Session 3.2. In agricultural crops

Abstract: Green leaf volatiles (GLVs) are commonly emitted by plants under herbivore attack. A broad range of laboratory studies have shown that GLVs can be used by predators and parasitoids of these herbivores to find their prey or hosts. The aim of this study was to evaluate the function of GLVs in the attraction of parasitoid wasps to maize plants under field conditions. In an experimental field, we planted maize *lox10* mutants impaired in GLV biosynthesis alongside wildtype plants and *lox8* mutants, which are, similar to *lox10* mutants, impaired in the jasmonic acid pathway, but produce normal levels of GLVs. The plants were infested with herbivorous *Spodoptera frugiperda* larvae, an important pest of maize that abundantly occurs in the area. A week later, plants were harvested and larvae were recollected to determine the degree of parasitism. Overall parasitism levels were low, but contrary to expectations, tended to be highest on non-emitting *lox10* plants, implying that GLVs were not of key importance for parasitoid attraction in the field.

Abstract: Stimulating plant defenses is a promising alternative method for limiting pesticide use in agriculture. To assess the defense status of the grapevine we have developed a triple approach called "BioMolChem".

- Biological tests measure the efficacy of grapevine defenses against two major biotrophic pathogens (*Erysiphe necator*, *Plasmopara viticola*).
- Molecular assays by q-RT-PCR show the expression patterns (over-expression or repression) of 24 genes involved in grapevine defenses, and they can then be correlated or not with the level of protection,

- BioChemical analyses of phenylpropanoides by HPLC are used to quantify and identify molecules of interest, and correlate them with specific gene expression (stilbene biosynthesis) and the acquired protection.

This tool was tested on leaves after stimulation by different elicitors (acibenzolar-S-methyl, phosphonates), on grapevine: Cabernet Sauvignon cv., resistant genotypes to powdery and downy mildew, and in the vineyard. We obtained correlations between the expression of various genes and with the level of protection. Similarly, we found correlations between the presence of known and unknown polyphenols and the level of protection. Resveratrol, a well-known phytoalexin of the grapevine, is a good marker of defense status but not of protection. Therefore, we now have an valuable tool for understanding the defense and protection status of the grapevine in laboratory and field experiments.

Abstract: The use of resistance inducers in crop protection is one of the most promising strategies that could be considered as either alternative or complementary to the use of conventional fungicides. However, the protection efficacy conferred by such inducers is often compromised in field conditions since their activity depends on many environmental factors. Here, we investigated the dose effect of four resistance inducers (referred as FSOV2, FSOV4, FSOV7 and FSOV10) on the protection of wheat against *Mycosphaerella graminicola*, the causal agent of *Septoria tritici* leaf blotch (SLB). Our investigations were performed on the susceptible wheat cultivar Alixan in both laboratory and field conditions. At the laboratory scale, each inducer was used on its own at four different doses. At the field level, each inducer was used at a single dose, on its own or in mixture with other inducers, and in association with the Cherokee® fungicide (50 g/l cyproconazole + 62.5 g/l propiconazole + 375 g/l chlorothalonil). A significant protection level was observed for all products, but the efficacy obtained clearly increased with the dose used. Further investigations revealed that all inducers exhibited at the highest dose only a direct *in vitro* effect on the fungus. A dose-dependent effect on host penetration and on the induction of peroxidase (PO) activity involved in plant defence mechanisms was highlighted for FSOV7 and FSOV10.

Session 4. Breeding for inducible resistance

Abstract: Knowledge about mechanisms for induced plant resistance, and susceptibility, to pests and diseases is rapidly increasing. Such knowledge is useful when breeding for inducible resistance that is aimed to be specific, efficient and with small costs to the plant. The inducing agents can be the pests or diseases themselves or resistance-inducing compounds. However, antagonism between plant defences to biotrophic and necrotrophic organisms is a problem that must be taken into consideration.

Extended abstract

Abstract: We have developed a high-throughput phenotyping system, in which a circulative virus (TuYV) serves as an indicator for *M. persicae* resistance. The aphid-virus system may be developed for other insect-virus combinations as well. Characterization of the mutants with increased resistance towards aphids led to the identification of novel genes involved in the mechanism.

Abstract: Maize is attacked in the field by three different feeding guilds of herbivores that include chewing, sucking and root feeding pests. The inbred, Mp708 appears to have resistance to each of these feeding guilds. Previous work has demonstrated its resistance to fall armyworm (FAW, *Spodoptera frugiperda*) and western corn rootworm (WCR, *Diabrotica virgifera virgifera*) and recently we have discovered that it is also resistant to the corn leaf aphid (CLA, *Rhopalosiphum maidis*). Our results have shown that transcripts for *mir1*, the gene encoding the cysteine protease Mir1-CP which is toxic to caterpillars, accumulate within 1 hr post CLA feeding. Preliminary results suggest that jasmonic acid (JA) and ethylene (ET) regulate *mir1* expression in response to FAW whereas ET alone regulates *mir1* expression in response to CLA. Work is underway to determine if western corn rootworm (WCR, *Diabrotica virgifera virgifera*) also affects *mir1* expression. Since the inbred Mp708 was developed from maize landraces that originated from Central America, we speculate that the parental landrace was selected because of its ability to resist herbivory in an area of high insect pressure and propose that the capacity to express *mir1* may have been lost during the modern breeding process. To test this we are studying the ability of a diverse group of maize genotypes to accumulate *mir1* transcripts in response to herbivory.

Abstract: Four stimulators of plant defences (SPD) were tested on two pathosystems: powdery mildew (PM) due to *Podosphaera xanthii* on melon and downy mildew (DM) due to *Bremia lactucae* on lettuce. More than 450 accessions of melon were screened for the stimulation of their defence reactions by a formulation with laminarin, BION® 50WG and CalFlux and more than 400 accessions of lettuce with BION, Calflux and a formulation with yeast extract (ABE IT 56). In the PM/melon pathosystem, only BION was able to induce resistance; in the majority of the accessions resistance was not activated but some accessions fully susceptible to PM in the control sprayed with water were very well protected by BION. In the DM/lettuce pathosystem, the three tested SPD could induce resistance, but again BION was the most effective. Close to half accessions of the 300 susceptible to Bl: 25 and a quarter of the 110 accessions tested with Bl: 26 were well protected against the tested strain by BION and some of these were also protected by CalFlux (13% and 14%, respectively) and few by ABE IT 56 (10% and 4%, respectively). In both pathosystems, the induced resistance seems to be strain-specific as some accessions were protected against one strain and not protected against another strain; nevertheless some promising accessions were protected against both strains.

Abstract: The induction of plant resistance could be a helpful alternative to conventional fungicides in order to protect in a more durable manner wheat crop against *Mycosphaerella*

Posters

Abstract: Tropical crop infestation by soil borne parasites (pineapple: *Rotylenchulus reniformis* and banana: *Pratylenchus coffeae*) cannot be controlled anymore by pesticides in French Antillas under the new European regulations. Therefore, we investigated the possible contribution of induced systemic resistances (ISR) to biocontrol pests and to develop more environmentally friendly agrosystems. In Martinique (Campus Agro-environnemental Caraïbe), we are presently testing a strategy based on current knowledge on ISR through interaction between plants and beneficial microorganisms. The investigations are based on four main hypotheses: 1) The inoculum of soil borne parasites can be reduced introducing non-host rotation plants. Cover crops were selected on the basis of several functional traits (non-host status, high biomass), for their contribution to a balanced microfauna in the rhizosphere, and for their positive effect on soil mycorhization potential. 2) The selection of pineapple and banana varieties able to develop ISR AND adapt their metabolism to environmental changes is essential. We found differential responses against nematodes to an ISR inducer (methyljasmonate, 10^{-4}M) in several pineapple and banana varieties and we are now searching for a relation with markers of plant adaptability to environmental changes (genes for cysteine-proteases and their inhibitors phyto-cystatins). 3) The successful development of ISR responses is dependent on the capacity of a plant to tolerate abiotic stresses (drought, temperature, salinity…) in addition to the pathogens. As ISR may also be part of the global adaptability of plant metabolism to tolerate abiotic stresses from their habitat, we investigate the possible links between stress level and a plant's capacity to induce efficient ISR against soil-borne parasites (*ongoing*). 4) Pineapple and banana root systems bear diazotrophic bacteria (endophytic) that can be used as ISR inducers in the field. Seventy-five and ninety-one diazotrophic bacteria strains (endophytic) respectively for pineapple and banana were isolated from their root systems in different sites in Martinique including organic and intensive cropping systems. They are currently being identified (MIDI – FAME and ADNr16S sequencing) and tested as ISR inducers. Our research aims at validating the hypothesis that efficient and consistent systemic resistances to pathogens can be achieved in the field by using selected varieties tolerant to abiotic stresses.

Session 5. Induced resistance and field adventures

Abstract: For a competitive and environmentally friendly agriculture, the enhancement of plant defence mechanisms by elicitor treatments seems a promising strategy. Since many years, elicitors have been explored in order to determine their efficiency towards plant diseases. Results under controlled conditions have been often very promising but the field reality has been very disappointing, with lower and less stable efficiency. In order to identify the barriers to elicitor development and find adapted solutions different actions have been taken lately. In 2006, a French network was created. Elicitra aims at understanding, developing and promoting this strategy for all crops. Many collaborative projects have also been started with interesting results.

Sub-Session 5.1. Field control of insect pests

Abstract: Greenhouse production of seedling transplants of leafy green vegetables usually requires the use of chemical sprays to avoid pest infestations that will be transferred and multiplied in the field. We investigated the use of trinexapac-ethyl (TE), a gibberellin synthesis inhibitor, as a seed treatment to induce resistance to whitefly (*Bemisia tabaci*, WF) in romaine-type lettuce seedlings grown in growth chambers or screenhouses. Leaves of young (2-3 wk old) lettuce seedlings grown from seeds treated with 1 mM TE had fewer WF eggs or nymphs, while older (4-5 wk old) seedlings had 30% fewer WF pupae and 50% fewer adults. Leaves of seedlings from treated seeds had greater amounts of carotenoids and anthocyanins, more antioxidative capacity, and thicker leaves and cuticular layers than those of controls. These TE-induced changes in leaf physiology or morphology provided resistance to WF attack and could reduce the environmental hazards of pesticide application.

Abstract: In addition to exploiting the inherited resistance in crop plants against the infesting insects, temporary induction of resistance against insect pests mediated through nutrient amendments is being explored now. Organic and inorganic sources of major and minor nutrients, when supplied to crop plants, influence and/or induce resistance against insect pests. In lieu of synthetic fertilizers bio-inoculants when amended to crop plants, offer resistance or at least tolerance to insect pests besides enhancing yield parameters. Preliminary attempts were made to explore the role of bio-inoculants in inducing resistance in tomato and sesame against selected insect pests. Three bio-inoculants viz., Arbuscular Mycorrhizal (AM) fungi, Azospirillum and phosphobacteria were evaluated in comparison with Farm Yard Manure (FYM) and inorganic fertilizers for their influence on the incidence of the shoot webber and capsule borer, *Antigastra catalaunalis* Dup. (Pyraustidae: Lepidoptera) on resistant (IVTS 2001-26) and susceptible (IVTS 2001-26) sesame accessions under field conditions in Tamil Nadu, a southern peninsular state in India. In another semi-field study with tomato, these bio-inoculants were evaluated for their influence on incidence of tomato fruit borer, *Helicoverpa armigera* (Hubner). In a subsequent in-depth study, four species of AM fungi namely *Glomus fasciculatum*, *G. mosseae*, *Acaulospora laevis* and *Gigaspora margarita* were found to enhance resistance in tomato against the noctuid caterpillars, *H. armigera* Hübner and *Spodoptera litura* Fab., through biochemical alterations especially by elevating the phenol content of the foliage. Similarly, *Azospirillum* amended sesame plants resisted the damage by shoot webber, *A. catalaunalis* which was mediated through increased levels of phosphorus and potassium. The levels of resistance induced by these bio-inoculants were found to be varying in each crop. The results of these investigations envisage further analysis of precise biochemical and molecular aspects of the interaction of microbial inoculants, crop plants and the infesting insect pests.

Abstract: Systemic acquired resistance (SAR) is a plant self-defense mechanism against a broad range of pathogens and insect pests. Among chemical SAR triggers, plant and bacterial volatiles are promising candidates due to high effectiveness and the fact of being cheap chemicals with relatively low concentrations compared to agrochemicals. However, before large scale application in agriculture to manage diseases, high evaporation rates after application, plant growth alteration, and inconsistent effectiveness need to be considered as major pitfall. In this

study, we provide a new evidence of volatile organic compound (VOC)-mediated SAR against both a bacterial angular leaf spot pathogen *Pseudomonas syringae* pv. *lachrymans* and a sucking insect aphid *Myzus persicae* in the open field without changing plant growth until harvesting. Unexpectedly, the drench of two VOCs, 3-pentanol and 2-butanone to cucumber seedlings caused significant increase of numbers of ladybird beetle that is known as a natural enemy of aphid. The defense-related gene, *CsLOX* was induced in volatile treatmented plants indicating to trigger oxylipin pathway responding in the emission of green leaf volatile that recruit the natural enemy. Our results demonstrate that VOCs are good resources to prevent plant diseases and insect damage by elicitation of SAR even in the open field.

Sub-Session 5.2. Field control of diseases

Abstract: Plants can be primed for more rapid and robust activation of defense. Priming follows perception of molecular patterns of microbes or plants, recognition of pathogen-derived effectors, or colonization by beneficial microbes. However the process can also be induced by wounding or treatment with some natural or synthetic compounds (including certain fungicides and insecticides). The primed mobilization of defense is often associated with induced disease resistance, stress tolerance, and preservation of yield. Although the phenomenon has been known for decades, the molecular basis of priming is poorly understood. This article summarizes recent progress made in unraveling molecular aspects of defense priming that is the accumulation of dormant mitogen-activated protein kinases, chromatin modifications, and the activity of transcription coactivator HsfB1. It also illuminates the potential of defense priming for plant production in the field.

Abstract: A method called “BioMolChem”, based on a triple approach (biological, molecular and biochemical) enabled us to evaluate and compare the efficacy and the mode of action of potential elicitors. This method, developed under controlled conditions, was applied in natural conditions in the vineyard. Although stimulators of plant defences are effective and reproducible in laboratory conditions, this method appears *in natura* more difficult to control and usually leads to widely variable results. Attempts were made in the vineyard in 2011 on plots treated with elicitors to provide some answers regarding the development of these alternative or complementary strategies. Elicitation in the vineyard shows a high variability but nevertheless provides significant protection under strong disease pressure, although being less efficient than a fungicide.

Abstract: A mixture of naturally-occurring compounds inducing plant innate immunity has been formulated as PRB1 for its use in agriculture with the aim to control plant diseases. Field trials have been set up to validate its use both as foliar treatment agent and as a fertirrigation additive. Independent trials in several years show that, PRB1 acts as an elicitor when applied to garlic as a preventive treatment increasing both the fresh and dry weight of garlic in a dose dependant manner. Furthermore, in comparative experiments with other commercial inducers we found that PRB1 has best performance in organic garlic production. In onion treatment, the weight increase is higher when PRB1 was applied directly by drip irrigation. In pepper greenhouse trials PRB1 is compatible with the bio-control agents *Amblyseius swirskii* and *Orius laevigatus*. PRB1 can be used in a numbers of crops to reduce disease levels and prevent the development and spread of

pathogens, thus improving yield and quality in both integrated pest management and in organic agriculture.

Abstract: COS-OGA is a new elicitor that combines oligochitosan and oligopectates in presence of calcium ions. Elicitation of tomato plants with COS-OGA has been tested and resulted in leaf peroxidase activity increase, transcription of SA-associated defence genes and overexpression of PR, heat-shock and DNA/RNA remodelling proteins. Greenhouse and field trials confirmed the efficacy of the elicitor against powdery mildew on cucumber and grapevine.

Abstract: Elicitors are a new promising way of protecting plants against pests and diseases. Based on natural or synthetic products, these products induce plant responses that lead to plant resistance. Three elicitors which presented no direct effects were introduced into a phytosanitary program, alternated with chemical references used against powdery mildew (*Podosphaera xanthii*).

On this multisite study, a few elicitors combined with chemicals reduce powdery mildew frequency and severity on melon crops in some conditions. However, the most efficient elicitor, acibenzolar-S-methyl (ASM), can impact final yield and sugar content.

Posters

Abstract: To find agronomic alternatives to reduce the amount of copper used as fungicide in vineyards against *Plasmopara viticola*, we tested a method using soluble carbohydrates as resistance inducers, as already showed against insects in earlier work in semi-field and field conditions.

In semi-field conditions on the grapevine cultivar “Muscat de Hambourg”, foliar applications of water solutions of D-glucose, D-fructose and sucrose were compared, each one at 1, 10 and 100 ppm doses. Fructose at 10 and sucrose at 10 and 100 ppm were as effective against downy mildew as copper hydroxide applied at a dose equivalent to 600 g per ha.

Trials were performed in organic vineyards following the organic cultural and control systems, on several cultivars in three regions of France. D-fructose water solutions at the dose of 100ppm were added to copper hydroxide of which the quantities were reduced to 100 g per ha. Keeping the efficiencies of the authorized regional copper references, this allowed reducing 2 to 6 fold the quantities of copper applied over the season. The effects of associating D-fructose and copper against *P. viticola* were lower than the addition of their individual effects.

Abstract: The dynamics of interactions between plants and herbivores is a major subject in ecology. In particular, the levels of defense traits are highly variable among and within plant species, including the occurrence and concentrations of defensive metabolites during a plant's lifetime. In grasses, which include an important range of crop species, benzoxazinoid derivatives (BXDs) have been recognized as an important family of compounds mediating resistance to

antagonists such as insects, pathogens and weeds. We studied the role of inducible BXD in plant-insect interactions using two sets of maize lines: a bx1 mutant which expresses only low levels of BXDs and its wild type. Sixty plants of each line were grown within a maize field. Half of the plants were experimentally induced with methyl-jasmonate one day prior to an artificial infestation with egg clutches of *Ostrinia nubilalis* (European corn borer, ECB). In September, buffer plants surrounding the two maize lines were examined for symptoms of ECB damage. The results show that ECB larvae disperse more to neighboring plants when feeding on wild type plants than when feeding on bx1 mutants. Similarly, a higher proportion of larvae emigrated from induced than from non-induced plants. This suggests that BXDs stimulate larval dispersal, thereby increasing resistance at the level of individual plants. However, by spreading larval attack over several plants, an increase in resistance may potentially decrease yield at the field level.

Abstract: *Bacillus amyloliquefaciens* strain IN937a, which was previously known as thriving inside plant tissues and referred to as an endophyte, was reported to stimulate plant growth and induce systemic resistance (ISR) via its emission of volatile organic compounds (VOC). We investigated the ISR capacity of the VOCs collected from the strain IN937a against bacterial spot disease caused by *Xanthomonas axonopodis* pv. *vesicatoria* on pepper in the greenhouse. Among 18 bacterial VOCs, 3-pentanol was selected for further experiments. 3-pentanol was drenched onto four week-old peppers before transplanting in the field. Disease severity was assessed at seven days after pathogen challenge when infiltrated in the pepper leaves at 10, 20, 30 and 40 days post transplant (dpt). 3-pentanol treatment significantly increased ISR compared to control treatment at 30 dpt. Taken together; bacterial VOC 3-pentanol can be utilized as a trigger of ISR against a broad spectrum of pathogens under field conditions.

Abstract: The potent inducer of resistance β-aminobutyric acid (BABA) was tested to control bunch rot and virus infection in grapevine. The aim of our research was to determine the effective but non-toxic doses and the appropriate application time of BABA for influencing bunch rot in the field. We performed our studies on the grapevine cultivar Királyleánka, which has thin peeled berries with enhanced susceptibility to *Botrytis cinerea*. The treatments have been carried out with four different BABA concentrations (0.2, 0.4, 1.0, 2.0 g/l). The Botrytis infection rate in case of 2.0 g/l BABA-treatment was significantly lower than in the untreated control. The highest dose applied at BBCH 65 phenological state also proved to be effective on reduction of the compactness of the bunches. In another experiment developing leaves of BABA-treated vines originated from virus (GLRaV-1) infected plants became virus free. Therefore BABA could be an effective tool for regulation of grape bunch structure of cultivars with compact bunches and for moderation of grey mould (*Botrytis cinerea*) and virus infections.

Abstract: Plant defence elicitors are small molecules that trigger induced resistance pathways. While some are used as a standard treatment against bacterial and fungal pathogens (e.g. Probenazole), the underlying science to others is less well defined. A key question is whether elicitors can be used to control opportunistic plant bacterial pathogens that are prevalent in soil and so replace the only viable option in current use; copper oxychloride. Elicitors were tested

against opportunistic plant pathogens of broccoli (*Pseudomonas fluorescens*, *P. marginalis*, *Pectobacterium carotovorum*) and onion (*Burkholderia gladioli* pv *allicola*) in field and glasshouse trials, respectively. Elicitors had a beneficial effect in reducing the likelihood of infection in onions. Although reproducible trends were evident from the broccoli trials, there were no clear significant differences in the incidence of bacterial disease.

Abstract: Studies on using trade product "EM" on strawberry plantations and various methods of their application were conducted in 2011-2012. The experimental area was established at an organic farm with cv. Honeoye. The tests were arranged in a randomized plot design, and each plot was repeated four times. Foliar spraying treatments with EM and dipping of the roots in EM solution were performed. Observations of plant development were carried out, including the fresh weight of above ground plants parts and numbers of runners. Additionally, elicitation of plant defence and chlorophyll content were studied. The highest mass of fresh plants cut from treated pots was noted for 5% EM, applied in 6 treatments with 14 day intervals. The increase to 10% EM did not influence plant growth parameters. A similar trend was observed in the number of runners. Regardless of the frequency of treatments after repeated treatment with EM (foliar spraying and treating the roots of plants before planting) at the end of the growing season, ß-glycosidase activity decreased and the content of phenols increased in strawberry leaf cells. This suggests an activation of the plant defence system. However, in 2012, despite EM concentration and treatments frequency, elicitation of plant defense response was not observed. There was no effect of EM treatments on chlorophyll a + b content.

Abstract: The use of biological methods in biofertilisation and bio-pest control is essential for the development of agriculture which respects the environment. Various agents have been tested, including micro-organisms. *Trichoderma* spp., regarding their antagonistic properties against a broad spectrum of plant pathogens, are used the most. Thus, the effect of two Algerian isolates of *Trichoderma* spp. on growth parameters and crop yield of three varieties of tomato (*Lycopersicon esculentum* Mill.) has been studied. The results have shown that these two strains have stimulated the growth of tomato plants, including vegetative biomass, but with a predominance of strain TR4. Also, the study found the power of *Trichoderma* spp. to colonize roots and to remain in the culture substrate. In addition, this study showed the effect of elicitor *Trichoderma* spp. against *Tuta absoluta* (Povolny, 1994) ex (Meyrick, 1917). Indeed, the two strains have reduced lepidopteran infestation. The infestation was almost zero for treated plants compared to controls, and this for the three studied varieties.

Abstract: Within the scope of a small plot trial we have tried to include the use of Alginure in a control strategy against apple scab (*Venturia inaequalis*) in organic agriculture rules during 2012. After application of Alginure the plant started to activate defence mechanisms. We have tested different dosages of Alginure and different numbers of applications. The best results were obtained with 5 applications at doses of 4 and 5 l/ha. Observed efficacy on leafs was 68% in case of 4 l/ha and 91% in case of 5 l/ha. Efficacy on fruits was a bit higher – 95% in case of 4 l/ha and 90% in case of 5 l/ha.

Abstract: The biocontrol isolate FS-94 (*Fusarium sambucinum*) secretes elicitors of plant defenses that prevent formation of lesions on wheat leaves by the fungal pathogen, *Stagonospora nodorum*. *In vitro* experiments show that FS-94 also secretes substances that enhance and prolong efficacy of a fungicide, Folicur® (tebuconazole), against *S. nodorum*. We describe some plant defenses elicited and that the protective effect of FS-94 secretion functions under field conditions. Integrated wheat protection against *S. nodorum* by co-application of F-94 secretions and Folicur is discussed.

Abstract: The changes in the leaves of cucumber infested by spider mites after application of two biostimulants, Asahi SL and Siapton 10L, were studied as well as the development of two spider mite (*Tetranychus urticae* Koch) populations. The cultivation of cucumber plants cv. Aramis treated and not treated with biostimulants and their infestation with mites was conducted in the greenhouse conditions. The samples of leaves were collected for analysis after 6 weeks of experiments. The content of primary metabolites, proteins and sugars, as well as secondary metabolites, phenols, was estimated in the young and older leaves of experimental plants to calculate the ratio between them in different experimental conditions. The activity of peroxidase (POX) in the leaves of mite infested plants treated and not treated with biostimulants was also compared. The treatment of plants with biostimulants decreased phenol concentration in older leaves of both mite infested and not infested leaves. However, the young leaves of infested plants treated with Siapton SL had the highest concentration of phenols and low concentration of sugars as compared to control plants what resulted in low value of ratio sugars: phenols. High increase in peroxidase activity was found in all plants infested by spider mites. An application of both biostimulants on cucumber plants caused, however, significant increase in peroxidase activity as compared to not treated plants. The spider mite populations in all plants treated with biostimulants were lower than on control ones.