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“Induced resistance in plants against insects and diseases”

Methods in research on induced resistance and tolerance

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Method to study the resistance induced by spraying epiphytic yeast against an insect pest (*Cydia pomonella* L.)

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Abstract : Primary metabolites (sugars) of the phylloplane stimulate the egg laying of the codling moth, *Cydia pomonella*. By modifying ratios and quantities of these metabolites we can decrease the number of eggs laid on the plants and could modify the development of pathogens. We thus spray apple trees with an epiphytic microorganism, which may modify the phylloplane composition by its metabolism and/or by inducing plant resistance mechanism, and as a consequence, modify the insect egg laying. We first screened epiphytic microorganisms able to survive on apple plant parts and to cover the whole foliage. Then we treated apple trees with one of the selected microorganisms (a yeast), and looked at the effect on egg laying. The preliminary results show that the egg repartition within trees is not changed by the presence of introduced microorganisms, but the egg number laid on trees is reduced up to 60% by the treatment. The first results of the phylloplane chemical analyses showed that the treatment changed the ratios within the sugar blend.

Analysis of early events involved in signalling pathways leading to plant defense responses

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Abstract: Our work focused on characterisation of intracellular early events following elicitor treatment. Using tobacco and grapevine cell suspensions, we were able to monitor AOS and NO production, Ca influx and concentration variations and MAP kinases activation. These analyses allowed to establish in our models, the relationships between these events. They turned out to be useful to screen candidate elicitor compounds.

Apple tree resistance against an insect pest by an elicitor (ASM). Investigations by analyses of the leaf surface metabolites of the tree sites

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Abstract: By using the inducer Acibenzolar-S-Methyl (ASM), which has been shown as an inducer of systemic resistance of apple trees against *Erwinia amylovora,* we induced an antixenosis resistance against the insect pest *Cydia pomonella*, particularly against egg laying which was reduced by 60 %. To understand the process we had to look at the primary metabolites which are present on the plant surface, known as egg laying stimulant. We had also to differentiate sites within apple tree and particularly the bourse shoot leaves. By these two ways we observed that the treatment induced a reduction of quantities of metabolites present on plant surfaces, soluble carbohydrates and sugar alcohols and changed also their ratios. A negative relationship between sucrose and sorbitol quantities was observed on the bourse shoot leaves. Experiments of egg laying in laboratory on artificial substrates reproducing the sugar compositions of the upper bourse shoot leaf surface we reproduced egg laying reduction by 50% with high level of sucrose and low of sorbitol.

Investigating the ecology of inducible indirect defence by manipulating plant phenotype and genotype

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Abstract: A challenge for ecologists has been to understand how individual traits of organisms affect species interactions and community dynamics. Recent breakthroughs provide ecologists with delicate manipulative tools in which mechanistic knowledge of well-characterized genotypes and phenotypic plasticity can be exploited to study the effect of individual plant traits on interactions in ecosystems. Food webs are overlaid with infochemical webs that mediate direct and indirect interactions. It is increasingly clear that indirect interactions can have important effects on community dynamics. Infochemicals are interesting in this respect because they cannot be directly used in bodybuilding, yet the responses they elicit have important consequences for fitness, and thus for interactions in a community. Infochemicals from plants influence interactions with members of different trophic levels, such as carnivores and herbivores. The infochemical-phenotype of plants is plastic: infochemical emission is an active and specific process that is induced by herbivory. The infochemicals attract carnivores that affect the herbivore population. Additionally, the infochemicals also affect herbivore behaviour and characteristics of neighbouring competitor plants. Careful manipulation of the phenotypically plastic emission by plants provides unique opportunities to investigate the effect of the infochemicals on food-web interactions. This novel approach creates an essential link between molecular, chemical, behavioural and community ecology.

Cell death or not cell death: two different mechanisms for chitosan and BTH antiviral activity

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Abstract: Two plant activators, benzothiadiazole (BTH) and chitosan, have been used in this study, comparing their efficacy and mechanisms of action in the *Phaseolus vulgaris*/tobacco necrosis necrovirus (TNV) system.

Both compounds induced local (LAR) and systemic acquired resistance (SAR) to TNV, though to a different extent. Furthermore, SAR was fully established after different induction times in the two systems, namely 7 days for BTH and 2-4 days for chitosan.

Histo-cytochemical investigations showed that BTH treatments raised H_2O_2 level homogeneously in the leaf tissues, without triggering cell death. In parallel, peroxidases activity, which regulates H_2O_2 homeostasis, was evenly enhanced as well, accounting for the high reduction in both size and number of lesions caused by TNV challenging inoculation. Instead, chitosan treatments induced numerous callose deposition sites, followed by a network of micro HR-like lesions formed by small groups of dead cells in the palisade mesophyll. DAB staining showed that micro-lesions are the consequence of localised H_2O_2 accumulation, and in turn, of localised micro-oxidative bursts. Thus, it is likely that chitosan-induced micro-lesions are responsible for the observed high local resistance, meanwhile generating signals for the induction of SAR.

Elucidating the role and regulation of callose in BABA-induced resistance

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Abstract: Priming plants for enhanced callose deposition has emerged lately as one of the major mechanisms of resistance against necrotrophic fungal pathogens. We have previously shown that the resistance inducer β-aminobutyric acid (BABA) primes for callose accumulation after pathogen attack and that this effect can be totally reversed through the application of the callose inhibitor 2-DDG. To further investigate the role of callose in resistance we have tested the callose deficient *pmr4* (powdery mildew resistant-4) mutant. *pmr4* does not show BABA-induced resistance (IR) against *Plecto-sphaerella cucumerina*. Additionally, our results demonstrate that *pmr4* is resistant to *Pseudomonas syringae* and displays a constitutive expression of PR-1. Although it is known that BABA can prime for PR-1 expression this is not the case in *pmr4* since here the SA pathway is constitutively activated and thus it has already a high PR-1 expression level. *pmr4* is also impaired in BABA-IR against the necrotrophic fungus *Alternaria brassicola* due to the lack of callose accumulation upon infection or BABA treatment. In the case of the oomycete *Hyaloperonospora parasitica pmr4* shows a resistant phenotype. Here, the lack of callose rescues the repression of the SA pathway. To elucidate the role of callose in BABA-IR against *H. parasitica* and its relation with the SA pathway we tested the double mutants *pmr4-npr1* and *pmr4-pad4* which are blocked in different steps of SA pathway. Both showed wild type phenotype against the oomycete and were also protected by BABA. In contrast, the double mutant *pmr4-Nah*G was hypersusceptible to the *H. parasitica* and could not be protected by BABA. BABA-IR against *H. parasitica* is usually attributed to an activation of callose deposition, priming of the hypersensitive response and trailing necrosis. We observed that the double mutant *pmr4-npr1* is still able to accumulate more pathogen-induced callose upon BABA treatment and also shows induced trailing necrosis as observed in the *pmr4-pad4* mutant.

Based on our results we conclude that priming for callose deposition is essential for BABA-IR against *A. brassicicola* and is an important, but not the unique mechanism for BABA protection against *H. parasitica* since the induction of trailing necrosis can also contribute to stop the pathogen. Additionally, *NPR1* and *PAD4* seem not to be essential for trailing necrosis induction and the resulting resistance, while *Nah*G and *pmr4* mutations abolish BABA-IR against *H. parasitica*.

Methods to study the role of individual volatile organic compounds (VOCs) in indirect defenses of plants against herbivorous arthropods

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Abstract: In response to an attack by herbivorous arthropods plants emit complex blends of VOCs that are highly attractive to natural enemies of these herbivores. Rapid progress in physiological and molecular aspects of such indirect defense mechanisms in plants provides detailed knowledge of the induction and release of plant VOCs. Understanding of the importance of individual VOCs within complex blends for attracting natural enemies is, however, still rudimentary. Using the tritrophic system: maize plants (*Zea mays*), lepidopteran larvae *Spodoptera littoralis* and the parasitoids *Cotesia marginiventris* and *Microplitis rufiventris*, we present methods that have allowed us to generate and modify herbivore-induced VOC blends of known composition (experimental approach). Such blends were compared and tested for attraction to the wasps in olfactometer studies. In addition we describe a statistical method based on linking odor profiles of different maize inbred lines with wasp behavior to evaluate the attractiveness of individual VOCs (explorative approach). The combination of these approaches provides new insights in the relevance of individual VOCs involved indirect defenses of plants against herbivorous arthropods.

Induced resistance to *Fusarium* head blight in winter wheat

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Abstract: Development of *Fusarium* head blight (FHB) was studied in winter wheat pre-treated with potential defence activators. Several chemicals were pre-screened for their capacity to reduce development of *Microdochium nivale* in a detached leaf assay. Selected compounds were further tested for their capacity to reduce *Fusarium culmorum* development in heads of winter wheat in greenhouse and field experiments. In the detached leaf assay, leaves from plants pre-treated with a foliar fertilizer displayed reduced disease development compared to untreated control. A significantly reduced disease development of FHB in plants pre-treated with the foliar fertilizer was also registered in the greenhouse and field experiments. In the field experiment, harvested grains from plants treated with the foliar fertilizer had up to 75% reduction in *Fusarium* infected seeds compared to grains from non-treated plants.

Reverse genetic methods in research on induced resistance of grapevine: development of a vector for shRNA production to induce gene silencing

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Abstract: ß-aminobutyric acid (BABA), a non-protein amino acid is able to induce resistance in Arabidopsis plants through the priming of the salicylic acid (SA)- and abscisic acid (ABA)-dependent defence signalling pathways. BABA-induced resistance (BABA-IR) was also observed in grapevine against downy mildew. Treatments of the susceptible variety Chasselas with either benzothiadiazole (BTH, a SA analogue) or ABA, however, did not lead to protection while jasmonic acid (JA) treatment was able to induce resistance. Mutant screening to determine the importance of the different signalling pathways in BABA-IR was not feasible in grapevine, in contrast to Arabidopsis. Therefore we had to use alternative methods such as co-application of specific inhibitors together with BABA. This type of approach yielded results suggesting that callose deposition and other defence mechanisms depending on the phenylpropanoid and the lipoxygenase pathways all contributed to BABA-IR. Expression patterns of marker genes, on the other hand, indicated the priming of both the SA- and JA-signalling pathways in BABA-treated Chasselas plants. These results point to major differences in the expression of BABA-IR in Arabidopsis and grapevine. In order to determine the key components of BABA-IR specific for grapevine, we plan to use gene silencing techniques. While testing different techniques of siRNA production (transgenes encoding long hairpin-forming RNAs, dsRNA produced in E.coli), we opted for the development of a new system based on the U6 hairpin cloning system of Promega (siSTRIKE) and adapted for grapevine. For this purpose we cloned and sequenced a *Vitis vinifera* U6 promoter and used it to construct a new vector to produce short hairpin RNA (shRNA) in grapevine cells.

Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants

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Abstract: In response to feeding by herbivorous insects many plant species produce volatile compounds, particularly terpenoids, that are used by natural enemies of the herbivores to locate their prey. We are studying the factors that regulate this volatile production in cucumber (*Cucumis sativus*) leaves upon feeding by the two-spotted spider mite (*Tetranychus urticae*) and that are used as cues by the predatory mite *Phytoseiulus persimilis*. Cucumber shows a clear and specific induction of volatiles upon spider mite feeding or jasmonic acid spraying. Here we show how we use metabolomics and transcriptomics to isolate and characterize enzymes and genes involved in signaling and volatile production.

Induction of resistance against tomato powdery mildew *(*Leveillula taurica*) by* Acremonium alternatum

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Abstract: *Acremonium alternatum* reduced powdery mildew infection by *Leveillula taurica* on tomato leaves and on cucumber cotyledons when spores were applied alive or killed. The effect was systemic, protecting untreated leaves above the treated ones and depended on temperature and leaf age.

Induction of defence related enzymes and systemic resistance by the plant activator acibenzolar-S-methyl in sugar beet against *Cercospora beticola* Sacc.

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Abstract: The involvement of systemic acquired resistance in Cercospora leaf spot control by the application of acibenzolar-S-methyl (ASM) was studied under greenhouse and field conditions.

Nine plants of the sugar beet Monodoro cultivar, partially resistant to *Cercospora beticola*, were treated with ASM (Bion, Novartis) in the greenhouse and inoculated with the pathogen (T+I) four days later. Untreated – non-inoculated (C), treated – non-inoculated (T) and untreated – inoculated (I) groups of plants served as controls. Leaf samples of 300 mg per plant were collected 2, 4, 7 and 10 days after inoculation in order to analyse the induction of PR proteins peroxidase and chitinase after IEF in 3-5 pH range.

One peroxidase isoform (pI 4.7) was induced on days 7 and 10 in T+I and T plants, while it was absent in I and C plants. Three chitinase isoforms showed the highest enzymatic activity in T+I and T plants, reaching a peak 7 days after the inoculation.

In the open field, the same cv. was used, under a natural pressure of pathogen inoculum, comparing repeated ASM treatments with fungicide applications and untreated control.

The disease incidence of ASM treated plots was significantly lower than the control and not different from fungicide treated plots, showing the effectiveness of ASM treatments in Cercospora leaf spot control and in inducing plant resistance to the pathogen.

Control of phytopathogenic bacteria by chitosan

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Abstract: Conventional chemical and biological methods of plant protection are not sufficient in control of bacterial diseases thus, induction of plant defense mechanisms is a potential method to reduce phytopathogenic bacteria.

Chitosan, a natural copolymer of glucosamine and deacetylglucosamine shows wide biological activity and is biodegradable, biocompatibile and safe for environment. The aim of this work was to assess the effects of chitosan on the growth of pathogenic bacteria *in vitro* and on bacterial plant diseases. Antibacterial activity of chitosan derivatives was manifested dually: by the inhibition of the growth of bacteria *in vitro* and by the inhibition of bacterial infection.

Only cationic polymers of chitosan inhibited of the growth of bacteria, and cationic chitosan derivatives induced resistance of plant to bacterial infection. Efficacy of chitosan depended mainly on type of chitosan derivative as well as bacteria and plant species. Chitosan acted against bacterial diseases of plants more preventively than therapeutically.

Induced resistance with extracts of *Reynoutria sachalinensis:* crucial steps behind the scene

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Abstract: Plant extracts of *Reynoutria sachalinensis* are inducing resistance and tolerance in a variety of crops. Extract application leads to effective disease control of powdery mildew fungi on e.g. cucumber, tomato or grape vine as well as against e.g. *Botrytis cinerea* on young ornamental or vegetable plants.

The induction by *R. sachalinensis* is characterised by a variety of processes following treatment with the extract and the pathogen, of which some could be identified as crucial steps with respect to the disease control properties. In non-infected cucumber leaf discs, treatment with the extract resulted in the development of reactive oxygen species. Six hours after treatment, levels of hydrogen peroxide were increased from 3 µM in water treated leaf discs to 22 µM in extract treated. When in addition to the treatment with *R. sachalinensis* Plantacur E (formulated vitamin E preparation) was infiltrated into leaves, the level of hydrogen peroxide reached 11 µM, while Plantacur E applied alone did not induce any increase in H_2O_2. Plantacur E treatment of plants before the extract application reduced the efficacy of *R. sachalinensis,* indicating that the development of reactive oxygen species plays a major role in this induction process. In barley coleoptiles treated with *R. sachalinensis* extract, increased papilla formation was identified at the penetration sites of *Blumeria graminis* f.sp. *hordei* together with the accumulation of hydrogen peroxide in the papillae.

Enhanced activities of enzymes belonging to the phenolic pathway, which are involved in the production of phytoalexins, were qualitatively and quantitatively determined in cucumber plants infested with *S. fusca* and treated with *R. sachalinensis* extract. Treatment of conidia of *S. fusca* with these phytoalexins resulted in a significant decrease in germination. C-glycosyl flavonoid phytoalexins were proven to be responsible for collapse of *S. fusca* colonies. Possibly, these are also responsible for the vacuolisation of powdery mildew haustoria in cucumber leaves treated with *R. sachalinensis* extract.

The results show that different crucial steps can be linked to the disease control properties involved in resistance induced by *R. sachalinensis* extract. Important plant responses occur directly after extract treatment (priming), as well as at a later stage, i.e. after pathogen attack.

Silicon as inducer of resistance in tomato against *Ralstonia solanacearum*

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Abstract: Bacterial wilt is widely distributed in tropical, subtropical and some temperate regions of the world. Control of the causal agent, *Ralstonia solanacearum*, is difficult and host plant resistance breakdown was frequently observed. Therefore, only integrated control combining resistance and cultural and biological measures seems promising. Silicon amendment significantly reduced bacterial wilt incidence for tomato genotypes L390 (susceptible) by 26.8% and King Kong2 (moderately resistant) by 56.1% compared to non-treated plants grown in hydroponic culture. However, wilt incidence in silicon-treated plants of genotype L390 reached 100% at 13 days post inoculation, while in genotype King Kong2, final plant death was reduced by 20%. Bacterial numbers were significantly lower in silicon-treated compared to non-treated plants in King Kong2 at 2 dpi in midstems and in all organs at 5 dpi, and in Hawaii 7998 (resistant) in all organs at 2 dpi. Increased tolerance was observed in genotypes L390 and King Kong2 with silicon treatment. Silicon accumulated in roots. Negative correlations between root silicon content and bacterial numbers of midstems in genotypes Hawaii 7998 and King Kong2 suggested an induced resistance. Indications for an influence of host genotype and silicon treatment on the phenotypic conversion of *R. solanacearum* strain To-udk2-sb from fluidal to non-fluidal colonies *in planta* were observed. This is the first report on the effect of silicon on a bacterial disease and in a silicon-non-accumulator plant.