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Cross-talk among herbivore- and pathogen-induced signal cascades

Induced Resistance in Plants - Molecular, Environmental and Practical Implications

Joseph Kuc..... 1

Abstract: Induced Systemic Resistance (ISR) in plants to pathogens and insects has likely been with us from the time plants appeared on earth and animal immune systems have been with us since they appeared. However, neither ISR nor animal immune systems have prevented serious epidemics and serious losses continue. Nevertheless, the defense systems in plants and animals are very effective for the survival of the species, but upon occasion they are ineffective as judged by human standards. A major question is why and how do the defense mechanisms become ineffective and how can we maintain and enhance their effectiveness? Resistance is the rule and susceptibility is the exception, but why the exception? Defense systems are layered and have increased in complexity in the process of evolution of complex life forms. These innate and layered defense systems are likely not lost during evolution and they can be activated and enhanced. Is ISR evidence for such layered innate systems? Defensins and protegrins are antimicrobial peptides found in plants and animals that are part of an innate immune system which evolved before antibodies and lymphocytes. Upon such innate systems have been added other systems that differ in defense compounds, their elicitation and specificity for recognition/response and signal transduction/transport. Thus, ISR can be elicited by pathogens and insects and compounds that are as structurally unrelated as proteins and simple organics and inorganics. The compounds associated with defense also differ widely in structure and complexity. Superimposed on these observations is the fact that resistance/susceptibility can be highly specific on the species and cultivar level.

Key words: specificity and nonspecificity, sensitization, application

Rhizobacteria-mediated induced systemic resistance in *Arabidopsis*

Corné M.J. Pieterse, Jurriaan Ton, Saskia C.M. Van Wees, Shu Hase, Karen M.

Léon-Kloosterziel, Bas W.M. Verhagen, Johan A. Van Pelt, L.C. Van Loon 9

Abstract: Selected strains of rhizosphere bacteria have been shown to reduce disease by activating a resistance mechanism in the plant called rhizobacteria-mediated induced systemic resistance (ISR). ISR resembles pathogen-induced systemic acquired resistance (SAR), in that both types of induced resistance render uninfected plant parts more resistant towards a broad spectrum of pathogens. The spectrum of effectiveness of ISR and SAR largely overlaps but is also partly divergent. In contrast to SAR, ISR induced by *Pseudomonas fluorescens* WCS417r is independent of salicylic acid (SA) and *PR* gene activation. Instead, ISR follows a signaling pathway in which components from the jasmonic acid (JA) and ethylene (ET) response are successively engaged to trigger a defense reaction that, like SAR, is controlled by the regulatory factor NPR1. To investigate the role of JA and ET in ISR, their production was monitored in ISR-expressing plants. Neither JA nor ET production changed upon induction of ISR. From this we postulate that ISR is mediated via an increase in the plants sensitivity to JA and ET. This is supported by the potentiated expression of the JA-inducible gene *AtVSP* observed in challenged, ISR-expressing plants. Moreover, preliminary results indicate that the ACC oxidase activity is enhanced in ISR-expressing plants, providing a greater potential to produce ET upon

challenge. In our search for ISR-related genes we identified two genes that show altered expression upon induction of ISR: the JA-inducible gene *AtVSP*, which shows an enhanced level of expression in challenged, ISR-expressing plants, and a root-specific, ET-inducible thaumatin-like gene, which is activated upon colonization of the roots with ISR-inducing rhizobacteria. Moreover, we identified a locus (*ISR1*) on chromosome 3 that controls the expression of ISR. *Arabidopsis* genotypes that are affected in this locus are also less sensitive to ET. Together, these data confirm the important role of JA and ET in ISR signaling. Cross-talk between SA- and JA-dependent pathways can result in inhibition of JA-mediated defense responses. For instance, chemical agents that activate the SAR pathway, e.g. SA and benzothiadiazole (BTH), can affect the JA-dependent wound response, which plays a role in defense against insects. We investigated possible antagonistic interactions between the SAR pathway and the ISR pathway. Simultaneous activation of SAR and ISR in *Arabidopsis* resulted in an additive effect on the level of induced protection against *Pseudomonas syringae* pv. *tomato*. In *Arabidopsis* genotypes that are blocked in either SAR or ISR, this additive effect was not evident. Moreover, induction of ISR did not affect the expression of the SAR marker gene *PR-1* in plants expressing SAR. Together, these observations demonstrate that the SAR and the ISR pathway are compatible and that there is no significant cross-talk between these pathways. Therefore, combining SAR and ISR provides an attractive tool for the improvement of disease control.

Key words : cross-talk, defense signaling, ethylene, ISR, jasmonic acid, salicylic acid, SAR

Arabidopsis defense against pathogens: a set of superimposed shields

Bart P.H.J. Thomma, Iris A.M.A. Penninckx, Willem F. Broekaert, Bruno P.A.

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Abstract: Not more than a decade ago it was generally accepted that defense mechanisms induced by micro-organisms converge into a central signalling cascade that regulates a defense response consisting of multiple components. Salicylic acid was recognized as a central regulator molecule in this signalling cascade. However, by now we know that the defense response of plants to pathogens is not regulated through a single signalling cascade, but rather through a complex network of different signalling pathways. These pathways are regulated through different signalling molecules. Since a considerable amount of cross-talk is observed it is clear that pathways are interconnected. As a model, we envisage the inducible *Arabidopsis* defense as a set of superimposed shields, each displaying their own efficacy against different pathogens.

Key words: salicylic acid, jasmonate, ethylene, camalexin, *Alternaria*, *Botrytis*, *Plectosphaerella*

Involvement of EDS1 and PAD4 at multiple levels of plant defense

Jane E. Parker, Bart J. Feys, Lisa J. Moisan, Marie-Anne Newman, Christine

Rustérucci, Danny Aviv, Jeffery L. Dangl..... 25

Abstract: We have used the model plant *Arabidopsis* to identify genes required for pathogen recognition (Resistance or *R* genes) and for activation of local plant defenses. Isolation of these genes and characterization of their functions has been undertaken by comparing mutant and wild-type plant resistance phenotypes, cloning and molecular analysis of the respective proteins. In particular, we have examined the roles of EDS1 and PAD4, two lipase-like proteins that positively regulate resistance mediated by a structural subset of *R* gene. They share certain motifs and interact in a yeast two-hybrid assay, suggesting that physical association may be important to their cellular functions. However, they have intrinsically different roles within the resistance pathway. Whereas EDS1 is required for elaboration of the localized hypersensitive response (HR) and its accompanying oxidative burst (producing reactive oxygen intermediates, ROI), PAD4 functions downstream or independently of these events. However, both EDS1 and PAD4

are required for accumulation of the SAR signalling molecule, salicylic acid (SA) in the *R* gene-mediated response. The genetic and molecular data lead us to position *EDS1*, in the absence of *PAD4*, as a regulator of early plant responses but together with *PAD4* in potentiation of plant defenses. In support of this model we find that *EDS1* and *PAD4* are equally required for basal resistance against several virulent pathogens. Moreover, *EDS1* and *PAD4* are necessary for signalling leading to SAR. Genetic epistasis studies also establish that *EDS1* and *PAD4* are essential components of runaway cell death in *lsl1* mutant plants that can triggered by pathogen inoculation or artificial provision of ROI. Thus, *EDS1* and *PAD4* may process an ROI-dependent signal in a defense potentiation loop. We are now examining *EDS1* and *PAD4* molecular associations in plant cells.

Key words: pathogen, plant defense, reactive oxygen intermediates, Resistance genes, salicylic acid, signalling

Activation of novel signalling pathways by phloem-feeding whiteflies

Wilhelmina van de Ven, David Puthoff, Cynthia LeVesque, Thomas Perring, Linda L. Walling

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Abstract: To investigate the signaling pathways induced by phloem-feeding whiteflies, tomato wound- and defense-response gene transcripts were evaluated after silverleaf whitefly (*Bemisia argentifolii*) and greenhouse whitefly (*Trialeurodes vaporariorum*) feeding. Temporal and spatial studies indicate that whiteflies are perceived as a bacterial or fungal pathogens and induce distinct gene sets from insects that cause extensive tissue damage. To identify novel genes that were induced in tomato and squash after whitefly feeding, differential RNA display was used. Genes expressed in apical, non-infested squash leaves after silverleaf whitefly feeding were isolated. *SLW1* (*SLIVERLEAF WHITEFLY-INDUCED 1*) and *SLW3* RNAs accumulated in apical, non-infested leaves (systemically) after feeding by silverleaf whitefly nymphs but not after feeding by silverleaf whitefly adults or sweet potato whitefly (*B. tabaci* Type A) adults or nymphs. Differences in *SLW1* and *SLW3* expression were also detected in infested leaves. *SLW1* RNAs were detected in flowers and fruit, while *SLW3* RNAs were not detected in any organ. Whitefly infestations did not alter this developmental programming. *SLW1* (M20b peptidase-like gene) and *SLW3* (a β -glucosidase-like protein gene) are modulated by different signaling pathways. *SLW1* RNAs accumulate in response to exogenous MeJA. In contrast, the defense signal modulating *SLW3* RNA levels is not known. *SLW3* RNA levels are not influenced by pathogen infection, wounding, infection, MeJA, ethylene, salicylic acid, ABA, or reactive oxygen species. Transgenic tomato plants expressing a *SLW3:GUS* gene will be used as bioassay plants to assess the whitefly-induced signal important in *SLW3* expression. Studies of tomato wound and defense response genes indicate that changes plant gene expression in response to whitefly feeding is distinct from aphids and caterpillars.

Key words: whiteflies, defense, systemic signaling, phloem-feeding, elicitors, saliva

Novel phospholipases A₂ induced during pathogen resistance responses in tobacco and with potential role in oxylipin biosynthesis

Thierry Heitz, Sandrine Dhondt, Guillaume Gouzerh, Pierrette Geoffroy, Michel Legrand

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Abstract : The induction of resistance of plants to infection by some microbes or against insect attacks is dependent on the production of fatty acid derivatives called oxylipins, whose best known representative is jasmonic acid (JA). Oxylipins display signalling or direct antimicrobial properties and may also affect cell death in the host. Their biosynthesis requires the enzymatic release of unsaturated fatty acids from membrane lipids, a rate-limiting step which remains elusive. This work reports on the isolation and cloning of the first phospholipases A that are induced after pathogen attack. Enzymes responsible for a strong increase in soluble phospholipase A₂ activity in tobacco reacting hypersensitively to tobacco

mosaic virus (TMV) belong to the patatin-like family of acyl hydrolases and may contribute to the massive lipid breakdown occurring during responses to pathogens.

Key words : jasmonate, lipid signal, oxylipin, patatin-like, pathogen defense, phospholipase A₂

Genetic dissection of induced resistance in tomato

Gregg A. Howe, Lei Li, Gyu In Lee, Chuanyou Li, David Shaffer..... 47

Abstract: We are using a genetic approach to dissect the mechanisms of wound signaling and induced anti-herbivore defense in tomato. Various screens were carried out to identify mutants that are defective in the systemic expression of the wound-responsive proteinase inhibitor (*PIN*) genes. Genetic analysis indicates that the mutants define at least five genes that are required for both systemic wound responses and the action of systemin, a peptide signal of the wound response. Phenotypic characterization suggests that the mutants can be classified as being defective either in systemin perception or JA biosynthesis and/or accumulation. The MicroTom dwarf cultivar of tomato is being used to facilitate genetic screens for new classes of wound response mutants. A mutagenized population of MicroTom was screened for plants that are insensitive to jasmonic acid (JA), a key regulator of induced resistance. Here we report the preliminary characterization of one such mutant that is blocked in JA perception and, as a consequence, is completely compromised in herbivore-induced expression of defense-related genes.

Key words : Induced systemic resistance; Jasmonic acid; Systemin; Wound response; Tomato

Ethylene insensitivity in tobacco and *Arabidopsis thaliana* affects resistance to soil-borne pathogens

Bart P.J. Geraats, Peter A.H.M. Bakker, L.C. van Loon 53

Abstract: The plant hormone ethylene can both affect the development of disease symptoms and enhance resistance in many plant-pathogen interactions. When transgenic ethylene-insensitive tobacco plants (Tetr) are grown in potting soil, they develop symptoms of wilt and stem base necrosis within 3-10 weeks, whereas the wild type plants remain healthy. From stem tissue of diseased Tetr plants, three oomycetes and four fungi were isolated that caused similar disease symptoms when inoculated on non-diseased Tetr plants. The isolates were identified as *Pythium sylvaticum*, *Pythium* sp. "group HS", *Pythium* sp. "group G", *Fusarium oxysporum*, *F. solani*, *Thielaviopsis basicola* and *Rhizopus stolonifer*. Ethylene-insensitive plants were more susceptible than wild type plants to six different *Pythium* isolates. Ethylene insensitivity also appeared to increase susceptibility to *T. basicola* and the two *Fusarium spp.*

Key words : *Arabidopsis*, ethylene, *Fusarium*, *Pythium*, resistance, *Thielaviopsis*, tobacco

Phenolic acids in tomato plants induced by carmine spider mite (*Tetranychus cinnabarinus* Boisduval) feeding

M. Kielkiewicz..... 57

Abstract: The objective of this study was to determine the concentration of phenolic acids in tomato leaves (*Lycopersicon esculentum* cv Slonka) that vary in the degree of their attractiveness to carmine spider mite (*Tetranychus cinnabarinus* Boisduval) and to examine if mite feeding damage induces changes in those phytochemicals in both infested and mite-free foliage from the same plant. Tomato plants were infested with 25 females per leaf. After 2 weeks of mite feeding, infested (middle) and mite-free (upper) leaves were sampled and analyzed, using HPLC techniques, for the concentration of phenolic acids. Caffeic, sinapic, ferulic, chlorogenic and vanillic acids were prevailing among derivatives of cinnamic and benzoic acids, both in the upper and middle tomato leaves. The upper leaves were significantly richer in those compounds, which to some extent might affect the feeding behaviour of *T. cinnabarinus* on these plants. In mite-infested leaves there was a greater increase of derivatives of cinnamic acid than of benzoic acid (gallic,

p-hydroxybenzoic, siringic, vanillic acids). It implies that derivatives of cinnamic acid, intermediates of the lignification pathway, are accumulated in cell protoplasts before converting to lignin. Ferulic and o-coumaric acids bound to the cell walls were the only phenolic acids markedly enhanced in mite-injured leaf. The increase of phenolic acids content in mite-damaged leaves was accompanied by the increase of gallic, p-hydroxybenzoic, vanillic, p-coumaric, ferulic and chlorogenic acids in mite-free leaves of upper part of the same plant. Results of this study clearly demonstrated that alteration in the concentration of two classes of phenolic acids in response to *T. cinnabarinus* feeding is not limited to the site of mite damage, but can occur systemically.

Fast assay to test compounds for their potential to induce PR-gene expression in grapevine (*Vitis spec.*)

Tobias Seibicke, Alexander Rügner, Gunther Neuhaus, Hanns-Heinz Kassemeyer, Günther Buchholz 63

Abstract: Plants respond to pathogen attack by activation of an array of inducible defense responses e.g. PR-proteins (*pathogenesis related*). Transcription of a β -1,3 glucanase gene was found to be induced in grape leaves upon infection with *Plasmopara viticola*. Promoter- and coding sequences of the gene were identified. In transient transformation assays the glucanase::GFP-fusion product was found to be localized exclusively in the cytoplasm of protoplasts derived from a *Vitis* cell culture. A fusion of the glucanase-promoter and the firefly luciferase-coding region was found to be induced by addition of salicylic acid (SA) in transiently transformed protoplasts. The same construct was used to create a stably transformed *Vitis* suspension cell culture for screening and detecting compounds acting as elicitors effectively inducing this PR promoter.

Key words: PR-proteins, glucanase promoter, luciferase, *Vitis*, plant cell culture, elicitor

Changes in secondary plant metabolites in cucumber leaves induced by spider mites and plant growth promoting rhizobacteria (PGPR)

Anna Tomczyk 67

Abstract: Changes in total phenols and cucurbitacin content were studied in the leaves of cucumber plants growing in the presence of PGPR in root system of healthy plants and plants infested with two-spotted spider mite (*Tetranychus urticae* Koch). In the leaves of cucumber plants variety Corona treated with bacteria as well as in mite infested plants an increase in phenol content was observed. In the leaves of cv Aramis the phenol increase was noticed only in bacterised plants, both infested and mite free.

High increase in total cucurbitacin content in the leaves of both studied cultivars of cucumber was found after spider mite feeding on nonbacterised plants and in the case of mite free, but bacterised plants. The combination of two factors - spider mites and PGPR - resulted in the decreased level of cucurbitacins as compared to separate influence of mites and bacteria.

Key words: *Tetranychus urticae*, cucumber, plant growth promoting rhizobacteria (PGPR), phenol content, cucurbitacin content

Identification of genes involved in rhizobacteria-mediated induced systemic resistance in *Arabidopsis*

Karen M. Léon-Kloosterziel, Bas W.M. Verhagen, Joost J.B. Keurentjes, L.C. Van Loon, Corné M.J. Pieterse 71

Abstract: Different forms of biologically induced disease resistance have been identified in plants. Following attack by a necrotizing pathogen systemic acquired resistance (SAR) is induced, leading to a broad-spectrum disease resistance that is associated with an increase in salicylic acid (SA) levels and the accumulation of pathogenesis-related (PR) proteins. Selected strains of non-pathogenic, root-colonizing fluorescent *Pseudomonas* spp. can

induce systemic resistance as well, without provoking any symptoms themselves. This rhizobacteria-mediated induced systemic resistance (ISR) is phenotypically similar to pathogen-induced SAR in that it is effective against various pathogens. In *Arabidopsis thaliana*, the ISR signaling pathway triggered by *Pseudomonas fluorescens* WCS417r requires responsiveness to both jasmonate (JA) and ethylene (ET) and is independent of SA and the accumulation of PR proteins. The state of pathogen-induced SAR is characterised by the concomitant activation of a set of PR genes. Of many defence-related genes tested in *Arabidopsis*, none were up-regulated prior to challenge in plants expressing WCS417r-mediated ISR. In an attempt to isolate ISR-related genes, we screened a large collection of *Arabidopsis* lines containing enhancer-trap *Ds* transposons and the β -glucuronidase (GUS) reporter gene with minimal promoter. One enhancer-trap line showed local GUS activity in the roots upon colonization with WCS417r. This local GUS expression was not observed after treatment of the roots with *Escherichia coli*, indicating that the induction was *Pseudomonas* specific. Interestingly, a similar expression pattern was observed after treatment of the roots with the ET precursor ACC, indicating that this line contains a transposon insertion in the vicinity of an ET-inducible gene that is up-regulated upon colonization with WCS417r. There are several candidate genes in the vicinity of the enhancer-trap *Ds* transposon, one of which encodes a thaumatin-like protein. Gene expression analyses confirmed that this thaumatin-like gene, designated *THL1*, is up-regulated in response to treatment of the roots with WCS417r or ACC. Analysis of the role of *THL1* in ISR might provide more insight into the molecular mechanisms involved in rhizobacteria-mediated ISR.

Key words : defense-related gene, ethylene, ISR, *Pseudomonas*, SAR, thaumatin

Prospects and challenges for practical application of rhizobacteria-mediated induced systemic resistance

L.C. van Loon, P.A.H.M. Bakker, C.M.J. Pieterse..... 75

Abstract: Selected strains of plant growth-promoting rhizobacteria are able to induce a systemic resistance (ISR) in plants, which is phenotypically similar to pathogen-induced systemic acquired resistance (SAR). The generally non-specific character of induced resistance constitutes an increase in the level of basal resistance to several pathogens simultaneously, which is of benefit under natural conditions where multiple pathogens may be present. ISR has been shown to be effective in radish and cucumber under field conditions. However, when induced plants are infected, disease development or severity are reduced but not prevented. Resulting economic losses to farmers make induced resistance alone insufficiently attractive for commercial application in modern intensive agriculture. For practical applications, durable strategies may be devised in which the growth-stimulating properties of resistance-inducing rhizobacteria are combined with other bacterial mechanisms of disease suppression through mixtures of rhizobacterial strains or by combinations with biocontrol fungi, low doses of chemical crop protectants, chemical SAR inducers or partial resistance. In *Arabidopsis* ISR and SAR are effective against a different, though largely overlapping spectrum of pathogens, depending on the signaling pathways involved in basal resistance. Combination of ISR and SAR can increase protection against pathogens that are resisted through both pathways, as well as extend protection to a broader spectrum of pathogens than ISR or SAR alone.

Key words : *Arabidopsis*, biological disease control, induced systemic resistance, plant growth-promoting rhizobacteria, *Pseudomonas*, radish, systemic acquired resistance

Induced responses by plant extracts from *Reynoutria sachalinensis*: a case study

Annegret Schmitt 83

Abstract: Plant extracts from *R. sachalinensis* induce local resistance in a variety of crops and against different plant pathogens. In cucumber, tomato and grape and in ornamentals

infection with powdery mildew or grey mould can be reduced to a large degree by regular application of the inducer.

Since treatment with this extract leads to changes in the plant metabolism and since the effects are depending on the crop plant, a number of investigations are necessary before the inducer could be applied in practice. Besides reduced infestation with pathogens, higher yields were recorded and morphological changes, such as darker green of leaves or enforced main shoot and flower production were found. Induced effects can however, not only bare “desirable” reactions, but can also lead to “un-desirable” effects. However, the definition of “un-desirable” is in many cases depending on the demands of the growers or the market. For use in practice, determination of the necessary concentration depends on the inducibility of the plant and performance can depend on the properties of the varieties of a crop or ornamental.

The borderline between induced resistance and induced tolerance is fluid. This means that a plant that still shows symptoms of disease can nevertheless produce e.g. a high quality and high amount of fruit because of the plant strengthening potential of the inducer. Therefore, i) research on induced resistance with respect to their practical application should be enforced, including all available information that comes from basic research, and ii) farmers need to be educated to assess the potential and effectiveness of an inducer in a different way than for non-inducing plant protection agents.

Key words : induced resistance, induced tolerance, Milsana, *Sphaerotheca fuliginea*, *Oidium lycopersicum*, *Botrytis cinerea*

Costs of induced resistance – what do we know and what can they explain?

Martin Heil 89

Abstract: The concept of fitness costs has been used to explain why defensive traits that benefit plants are still subject to variation in natural populations, instead of being fixed genetically at a maximum level. More resistant plants are predicted to have a lower fitness as compared to less well resistant plants, when both are compared under enemy-free conditions. This concept can also explain the occurrence of induced resistance, which appears only when plants are attacked. Some studies have presented results which hint to costs of induced resistance. However, all suffered from methodological problems. Some removed leaf material to induce resistance, others used chemical induction. While removal of leaf material can incur costs by itself, the latter suffer from the fact that resistance elicitors induce several physiological and morphological changes which are not related to resistance, but which may nevertheless affect fitness parameters.

No study has yet clearly proven that induced resistance against herbivores or pathogens can cause evolutionary relevant costs. However, the concept of costs seems to be the most powerful one to explain natural variability in constitutive resistance and the evolution of induced resistance. Both theoretical and experimental efforts should be spent on solving the question whether induced resistance causes costs at all and under which conditions these can lead to fitness reductions which might be of evolutionary relevance.

Key words: fitness costs, induced defence, ISR, indirect defence, herbivore, pathogen

Benefits and costs of induced volatile production in maize plants

Maria Elena Fritzsche Hoballah, Ted J. C. Turlings 95

Abstract: Herbivore-induced plant volatiles have been suggested to function as indirect defense signals that attract natural enemies of herbivores. Several insect parasitoids are known to exploit such plant-provided cues to locate their hosts. Maize plants can be induced to produce the volatile attractants by treating them with regurgitant of *Spodoptera* species. These artificially induced plants can be just as attractive to parasitoids as the complete plant-herbivore complex. We conducted a series of experiments to evaluate if plants indeed benefit from attracting parasitoids. It was found that the seed production of

maize plants after an attack by a parasitized caterpillar was higher than for plants attacked by a healthy caterpillar. We also showed that artificially induced volatile production in maize plants had some effect on plant development, but not on seed production. Our results suggest that benefits outweigh the costs when plants can successfully attract parasitoids with herbivore-induced volatiles and that the plant signals may be exploitable for crop protection.

Key words : *Zea mays*, *Cotesia marginiventris*, volatiles, induced resistance, integrated pest control

Time course of induced volatile emission of mature fruits upon herbivory, and response of conspecific adult herbivores and of a natural antagonist

Silvia Dorn, Alan Hern, Letizia Mattiacc 99

Abstract: The majority of herbivore induced plant volatile studies deal with phyllophagous herbivores. We studied induction by a carpophagous herbivore, *Cydia pomonella*, a key pest of apple orchards worldwide. Like many other carpophagous species, this insect is particularly critical in terms of ecological consequences as it penetrates as a larva into the apple fruit, proceeds to the center and eats the seeds.

We investigated the response of apple fruits to the infestation of the codling moth larva over its complete larval development period. The initial induction was short lived. This phenomenon observed in detached fruits might be related to the high metabolic cost of the induced process.

The volatiles emitted from mature fruits, mainly esters, a few aldehydes, and the terpene α -farnesene have an effect on approaching conspecific females. They were repelled in a bioassay with a linear tube olfactometer. Further, a parasitic wasp of *C. pomonella*, *Hyssopus pallidus*, discriminated preferentially in favour of infested fruit when given the choice with healthy fruit. Females spent more time searching on an infested apple than on a healthy apple. This indicates a potential ecological role of the herbivore induced volatiles in favour of the apple tree.

Key words: apple volatiles, induced response, *Cydia pomonella*, carpophagous insect, endophytic host, time course

Incidence of potato late blight assessed on stems and lower hierarchic levels can distinguish heterogeneous disease intensity effected by host reaction and application of salicylic acid and benzothiadiazole

Diego Fernando Meza, Enrique Torres 103

Abstract: Reliable estimates of disease intensity, measured as severity or incidence, are decisive for successful research programs of any crop disease and crucial when using control tactics that are expected to reduce the rate of disease progress. Potato late blight is a typical case, both when breeding for partial or field resistance, and when testing biological or chemical inducers of systemic resistance. Such reliability is hard to achieve in late blight when using severity, because of the variable expression of symptoms on leaflets, leaves, petioles and stems. On the other hand, disease incidence can be measured with greater ease and repeatability than severity, but its application to assess potato late blight has not been sufficiently studied. In this work, we examined the methodological and statistical validity of late blight incidence in plants, stems, leaves, leaflets and petioles as a quantitative measure of the disease. Three potato cultivars were grown in an environment prone to late blight epidemics and exposed to chemicals known to enhance natural plant defenses, with the purpose of modulating epidemics of varying intensity. Incidence data on the above mentioned structures were examined by analysis of variance of maximum accumulated incidence (y_{max}) and area under disease progress curve (AUDPC) and by rate of disease progress using five common population growth models. All analyses showed that incidence data can discern the heterogeneous levels of late blight brought about by the combinations of chemical treatments and host reactions. The most powerful statistical approach procedure was the use of growth

models, and the easiest and most convenient field method was to assess blight incidence on stems and leaves. These results show that incidence is a practicable alternative to severity in assessing the effect of treatments that slow potato late blight progress.

Key words: pathometry, integrated disease control, systemic induced resistance, epidemiology

Constitutive and induced variation in plants: what triggers the interaction between the polyphagous insect *Helicoverpa armigera* (Lep.: Noctuidae) and its antagonist, the baculovirus HaSNPV?

Annette Herz, Annegret Schmitt, Jürg Huber 107

Abstract: Potential effects of food plants of the polyphagous insect *Helicoverpa armigera* Hübner (Lep., Noctuidae) on the infectivity of the baculovirus HaSNPV were studied by considering the following scenarios: (1) effect of different food plant species, (2) effect of a previous infection of the food plant with a phytopathogen, (3) effect of a previous treatment of the food plant with the botanical inducer *Reynoutria sachalinensis* (F. Schmidt) Nakai and (4) effect of a previous infestation of the food plant with a herbivorous insect. The virus was fed to neonate insect larvae on excised leaf disks of test plants in dose-response-bioassays. A remarkable effect on virulence was only found, when the baculovirus was applied on the foliage of different food plant species of the host insect. Consequently, plant effects of the interspecific level are obviously more important for the considered insect - baculovirus system than those of the intraspecific level.

Key words: baculovirus, induced resistance, tritrophic interactions

Variation of C/N ratio and total protein content in *Cacopsylla*-infested leaves of three pear cultivars: relationships with an induced phenolic compound

P. Scutareanu, A. Johansson 111

Abstract: Previous experiments have shown an intraspecific variation in response to *Cacopsylla* feeding upon leaves of pear trees: a phenolic compound, previously identified as 3-O-trans-p-coumaroyl tormentic acid, is *de novo* and locally induced only in infested leaves of the European pear cultivar 'Conference', whereas it is constitutively expressed and increases in synthesis in the whole plant in two other pear cultivars, 'Bartlett' and 'NY10355', from North America. The results presented in this paper provided additional evidence for a difference in response to psyllids infestation among the three pear tree cultivars regarding the total protein content, but not C/N ratio. The relationships with the induced phenolic are discussed. It is suggested that there is a variation among pear cultivars regarding the nutrient/allelochemicals balance and consequently in the quality of the leaves as food for herbivores, which may influence the distribution of the herbivore populations in a pear orchard.

Key words: C/N ratio, induced response, pear cultivar, *Cacopsylla*, herbivore, protein, phenolics.

General aspects of induced resistance and induced tolerance

β -aminobutyric acid as a useful tool to dissect the priming phenomenon in induced resistance

Valérie Toquin, Gabor Jakab, Muriel Nirina Maeder, Brigitte Mauch-Mani 117

Abstract: Following induction of an induced resistance state in plants, defense pathways are activated at different levels. Depending on the inducing treatment, either induction of defense genes such as PR proteins associated with a faster resistance reaction of the plants can be observed or only an accelerated reaction of the defense pathways is apparent; this latter case is known as priming. Priming has been observed in several systems of induced resistance but the underlying molecular mechanism is still poorly understood. The chemical

inducer BABA has been shown to prime disease resistance without direct induction of PR genes in *Arabidopsis* and is therefore an ideal tool to analyze the process of priming. Using this approach, several BABA insensitive mutants have been identified and a preliminary characterization is presented.

Key words: *Arabidopsis*, BABA, conditioning, potentiation, mutant screen, T-DNA, plant defence

Priming as a mechanism in plant systemic acquired resistance

Uwe Conrath, Oliver Thulke, Vera Katz, Sandra Schwindling, Annegret Kohler 125

Abstract: Systemic acquired resistance (SAR) is a plant defense state that is associated with an enhanced capability - the so-called 'priming' - for stronger activation of cellular defense responses. So far, little is known about the molecular biology of priming. Over the past years, it has been demonstrated that pretreatment of cultured parsley cells with the SAR inducers salicylic acid (SA) or benzothiadiazole (BTH) leads to the direct activation of certain defense-related genes and also primes the cells for stronger elicitation of other defense genes including those encoding phenylalanine ammonia-lyase (PAL). From these results, it was concluded that resistance inducers have a dual role in plant defense gene activation. When elucidating whether priming plays a role in SAR of *Arabidopsis*, pretreating plants with BTH was found to augment the subsequent activation of PAL genes induced by *Pseudomonas* infection, wounding and osmotic stress and to also enhance wound/osmotic stress-induced callose production. The enhancement of PAL gene activation or/and callose deposition was not seen in the *Arabidopsis* non-expresser of pathogenesis-related genes (*npr1*) mutant which is compromised in SAR, while it was present, without BTH pretreatment, in the constitutive expresser of pathogenesis-related genes (*cpr1*) and *cpr5* mutants, in which SAR is constitutive. Together these studies suggest that priming is an important cellular mechanism in SAR of plants which requires the intact NPR1 gene.

Key words: *Arabidopsis*, benzothiadiazole, defense response potentiation, parsley culture cells, pathogenesis-related (PR) proteins, priming, salicylic acid, systemic acquired resistance

PR1a promoter activation and MAP kinase phosphorylation are involved in the stimulation of plant defenses by OxycomTM

Kris Blee, Kwang-Yeol Yang, Anne Anderson 129

Abstract: Commercial field use of OxycomTM is correlated with improved plant health in diverse crops. OxycomTM treatment induced expression from the PR1a promoter in tobacco leaves engineered with a PR1a promoter-GUS fusion. The response, detected by 9 h, increased in level up to 6 d and was accentuated by repeat applications. Corresponding increased formation of PRI protein was detected by antibody recognition. The promoters for other protective genes, peroxidase, osmotin and extensin, similarly were activated at 24 h. Activation of a MAPK preceded this response. Phosphorylation of a 48 kDa MAPK at the tyrosine and threonine active sites was observed after 10 min of treatment with OxycomTM of tobacco suspension cultured cells or intact leaves. The extent of phosphorylation decreased with time to 120 min. This same MAPK band was activated by salicylic acid and by hydrogen peroxide to a lesser extent. Similar transient activation of a MAPK detected by the Phospho-Thr/Tyr antibody was observed in maize callus, and *Arabidopsis* leaves. We propose that OxycomTM alteration of protective gene expression in plants involves at least the salicylic acid-MAPK pathway.

Key words: MAP kinases, systemic induced resistance

Induced resistance: an enhancement of basal resistance?

Jurriaan Ton, Sylke Davison, Martin De Vos, Charlotte Robben, Hans Van Pelt, L.C. Van Loon, Corné M.J. Pieterse 133

Abstract: Upon primary pathogen attack, plants activate resistance mechanisms at the site of infection. Besides this so-called basal resistance, plants have also the ability to enhance their defensive capacity against future pathogen attack. There are at least two types of biologically induced resistance. Classic systemic acquired resistance (SAR) results from infection by a necrotizing pathogen and is dependent on endogenous accumulation of salicylic acid (SA). Root colonization by non-pathogenic rhizobacteria can trigger an induced systemic resistance (ISR) response as well, which functions independently of SA and requires intact responsiveness to the plant hormones jasmonic acid (JA) and ethylene (ET). A screen for genotypes impaired in either ISR or SAR revealed that ecotypes RLD1 and Ws-0, as well as the enhanced disease susceptibility mutants *eds4-1*, *eds8-1* and *eds10-1*, are impaired in WCS417r-mediated ISR, whereas mutants *eds5-1* and *eds12-1* are impaired in pathogen-induced SAR. Analysis of JA-, ET-, and SA-responsiveness revealed that the ISR-impaired genotypes are affected in signaling compounds contributing to JA/ET-dependent basal resistance against *P. syringae*, while the SAR-impaired genotypes are affected in compounds contributing to SA-dependent basal resistance against *P. syringae*. To further examine the relationship between basal resistance and induced resistance, the effectiveness of SAR and ISR was assessed against different *Arabidopsis* pathogens that are resisted through JA/ET-dependent basal resistance, SA-dependent basal resistance, or a combination of JA/ET- and SA-dependent basal resistance. This analysis revealed that ISR is effective predominantly against pathogens that are resisted through JA/ET-dependent basal resistance, whereas SAR is effective against pathogens that are resisted through SA-dependent basal resistance. Collectively, our results suggest that ISR constitutes an enhancement of JA/ET-dependent basal resistance, whereas SAR is achieved through an enhanced expression of SA-dependent basal resistance.

Key words: *Arabidopsis*, resistance, jasmonic acid, ethylene, salicylic acid

Biological control of *Erwinia carotovora* subsp. *carotovora* on potatoes by fluorescent pseudomonads and *Bacillus subtilis*

A.I. Abdel-Alim, M.S. Mikhail, F.M. Barakat., P. Laux, W. Zeller 139

Abstract: Antagonistic bacteria were selected according to the production of inhibition zones on different media by using chloroform vapour method against *Erwinia carotovora* subsp. *carotovora* 429. From 69 antagonistic bacteria, we found that 12 bacteria produced inhibition zones on King's B medium against *E.c.c.429*. Two antagonistic bacteria were identified as *Pseudomonas fluorescens* biotype F (160 and 148) and one isolate as *Pseudomonas* spp. (E12) according to Biolog System. Four bacterial antagonists against *E.c.c.429*, *Bacillus subtilis* FZB 24 and three other isolates were tested on potato slices and also in the experimental field of the BBA. Isolates, 160 and E12 showed the best effect in the laboratory and field against *E.c.c.429*. Finally, we compared *Erwinia* species and also antagonistic bacteria by PCR based on 16S rRNA.

Key words : biocontrol, soft rot *Erwinia*, antagonistic bacteria, chloroform vapour method, PCR

Induced resistance in sunflower against *Orobanche cumana*

Holger Buschmann, Joachim Sauerborn 145

Abstract: This study evaluated the induced resistance to infestation of sunflower (*Helianthus annuus* L.) by the parasitic weed *Orobanche cumana* Wallr. Treatment of sunflower seeds with 30 and 60 ppm of benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) prevented infection in root chambers. In pot studies the total number of *O. cumana* was reduced up to 95 % in the 60 ppm BTH treatment. Here we report on defensive structures that may get preaccumulated in sunflower roots after BTH treatment but before infection. Chemical analysis of root extracts revealed *de novo* synthesis of secondary metabolites and H₂O₂ in the BTH treated sunflower roots. These results show that the phenomenon of induced resistance is not restricted to viral, bacterial and fungal

disease and demonstrate the great potential of this protection strategy as an effective component of future plant production systems.

Key words : Benzothiadiazole, BION[®], induced resistance, *Helianthus annuus*, *Orobancha cumana*, parasitic weed, SAR

Induced resistance in barley (*Hordeum vulgare* L.) against *Rhynchosporium secalis* and Barley Yellow Dwarf Virus (BYDV)

Claudia Weiskorn, Marco Krämer, Frank Ordon, Wolfgang Friedt 149

Abstract: The effect of SAR inducing chemicals on *Rhynchosporium secalis* and on barley yellow dwarf virus (BYDV) was determined in field, pot and greenhouse experiments on doubled haploid lines (DH lines). Regarding *R. secalis* a slight reduction of ELISA-values was measured after treatment with Dichloroisonicotinic acid (DCINA), jasmonic acid, and Bion[®]. However the results showed a high variability between the test series. Regarding BYDV a reduction of the virus titre after treatment with DCINA was measured by ELISA but no correlation between the reduction in virus titre and grain yield was found. In both pathosystems genotypic differences in the reaction to a chemical inducer treatment were present as in each DH population some lines react with an increase of ELISA value whereas most lines react with a reduction after treatment but without any correlation regarding the respective resistance/tolerance gene, i.e. *Rh* and *Ryd2*.

Key words: *Hordeum vulgare*, *Rhynchosporium secalis*, barley yellow dwarf virus (BYDV), systemic acquired resistance (SAR)

Benzothiadiazole enhances phenolic compound production and resistance to powdery mildew in strawberry

R.O. Karjalainen, A. Hukkanen, M. Anttonen, H. Kokko, S. Kärenlampi

K. Tiilikkala 155

Abstract: Induced resistance to pathogens is well-demonstrated in cereals and vegetables, but poorly known in berries. Powdery mildew is a serious disease in strawberry grown in plastic houses and tunnels in various parts of Europe. Disease control by chemicals is insufficient due to rapid development of insensitive strains of the fungus, and alternative control means are needed. We have tested the ability of benzothiadiazole (BTH) to activate plant defence mechanisms to powdery mildew in strawberry. As strawberry contains a variety of phenolic compounds implicated in protective function against plant diseases and health-promoting activities in human nutrition, we also tested the ability of BTH to increase the phenolic compound concentration in strawberry by analysed compounds by HPLC and ESI-MS-method. Treatments with BTH increased the levels of several phenolic compounds, particularly those of ellagic tannin, gallic acid, quercetin and kaempferol, but (+)- catechin and chlorogenic acid were not induced by BTH treatment. In berries, the levels of two flavonols, quercetin and kaempferol conjugates, were also increased in response to foliar treatments with BTH (60 g/ha) under field conditions. Treatments of young leaves before mildew contamination, effectively prevented the development of disease in greenhouse-grown strawberry. In the case of low-level mildew infection on leaves, repeated applications with BTH led to successful control of mildew in greenhouse.

The results suggest that BTH is not only capable of activating SAR-related genes, but also seems to induce a wide range of phenolic compounds in strawberry. BTH and related resistance elicitors may offer alternative means of powdery mildew control in strawberry grown in tunnels and greenhouses.

Key words: Strawberry, powdery mildew, elicitors, BTH, phenolics

Cloning of tomato proteases by direct selection in yeast for enzymes that cleave the polypeptide wound signal systemin

Jochen Strassner, Yoann Huet, Andreas Schaller 159

Abstract: A genetic screen in yeast was performed to identify proteases involved in either the maturation of the peptide wound hormone systemin from its precursor, or in the inactivation of systemin. Reporter gene expression was controlled by GAL4 rendered susceptible to proteolysis by insertion of the protease target sequence, i.e. systemin and part of the prosystemin sequence, between its two functional domains. Clones selected to confer loss of reporter gene expression included the cDNAs of three new tomato proteases, i.e. the proteolytic subunit of Clp proteases (*LeClpP*), a Xaa-Pro aminopeptidase (*LeAPP*), and a homologue of insulin-degrading enzyme (*LeIDE*). While all three proteases exhibited specificity for the target sequence, *LeClpP* and *LeAPP* were considered unlikely to be involved in systemin signalling and *LeIDE* was chosen for further study. *LeIDE* was expressed in *E. coli* as a glutathione *S*-transferase fusion protein and was purified from bacterial extracts. Recombinant *LeIDE* cleaved systemin carboxy-terminal of Lys14, resulting in systemin inactivation. Cleavage at this site was shown to be important for systemin inactivation *in vivo* and *LeIDE* is a candidate enzyme for such a function *in planta*.

Key words : wound response, tomato, systemin, protease, peptide hormone, insulinase

Plants' defensive responses towards insect oviposition

Torsten Meiners, Monika Hilker 165

Abstract: Plants have developed various reactions to insect oviposition ranging from changes of plant tissue to alterations of the plant's volatile bouquet. Various elicitors can induce these responses by employing different mechanisms causing diverse ecological effects, including direct and indirect defences. Compounds from the egg chorion or glandular secretion attached to the eggs can act as elicitor. Moreover, wounding of plant tissue by ovipositor valves or mandibles of egg-laying females might additionally be necessary. Egg depositions can cause tissue changes known as formation of neoplasm and hypersensitive response. Changes of the plant's volatile emission induced by insect egg deposition that cause the attraction of egg parasitoids or predators may positively affect the plant. We found that oviposition of a herbivorous insect induces the plant to release volatiles (synomones) attractive to egg parasitoids. The ability of plants to "call" for egg parasitoids as soon as eggs – the first life stages of herbivores – are touching the plant demonstrates the high complexity of insect-plant interactions. We expect that such chemically mediated plant defensive responses are also "listened to" by egg parasitoids or egg predators in other tritrophic systems.

Key words : oviposition, induced defence, elm leaf beetles, jasmonic acid, systemic effect

Immunohistological analysis of chemically induced proteins in sugar beet

Lenka Burketova, Katerina Stillerova, Marcela Feltlova, Milada Sindelarova..... 169

Abstract: Subcellular distribution of basic class IV chitinase (Ch4), class II chitinase (Ch2), acidic class III chitinase (SE2) and basic β -1,3-glucanase (Glu 2) were determined both in leaves and roots of sugar beet treated with salicylic acid (SA), benzothiadiazole (BTH) and glycine betaine. Protein localization was monitored by immunohistological analysis using a specific antibodies.

The most pronounced difference between induced plants and uninduced control was detected in leaves. In roots, low amounts of constitutively present proteins were found and their level did not increase after spraying with the inducers on leaves, but their synthesis increased when the inducers were applied directly on roots. High levels of Glu2 and Ch2 were observed in BTH treated, and Ch4 and SE2 in SA treated plants in extracellular matrix both in epidermal and mesophyll cells. Little stimulation of protein synthesis was found in glycine betaine treated plants. Induced proteins were spatially distributed over the whole plant regardless the site of the inducer application.

Key words: sugar beet, chitinase, β -1,3-glucanase, salicylic acid, benzothiadiazole, glycine betaine, immunolocalization

Induction of defence responses and snow mould resistance in cereals and perennial ryegrass

Åshild Ergon, Ingerd S. Hofgaard, Anne Marte Tronsmo 173

Abstract: The effect of cold acclimation and defence activators on snow mould resistance and expression of pathogenesis-related (PR-) genes were investigated in winter cereals and perennial ryegrass. Cold acclimation, known to induce snow mould resistance, had a potentiating effect on snow mould-induced PR-gene expression in winter wheat. Chitosan treatment induced chitinase expression and in some cases also snow mould resistance in winter wheat. BION treatment reduced snow mould resistance in perennial ryegrass.

Key words: BION, BTH, chitinase, chitosan, cold acclimation, cold hardening, *Lolium perenne*, *Microdochium nivale*, PR-proteins, *Triticum aestivum*, winter wheat

Extrafloral nectar produced by *Macaranga tanarius* is an induced, indirect defence against herbivores

Martin Heil, Thomas Koch, Andrea Hilpert, Brigitte Fiala, Wilhelm Boland, K. Eduard Linsenmair 177

Abstract Many plant species produce extrafloral nectar (EFN) on their leaves or shoots. Predators and parasitoids, such as ants and wasps, are attracted to this reward and in turn defend the EFN-producing plants against herbivores. Several experiments conducted in Malaysia showed that EFN secretion by field-grown *Macaranga tanarius* increased after herbivory, artificial leaf damage, and when jasmonic acid (JA) was sprayed on the leaves. Damaging leaves artificially with a needle strongly enhanced endogenous JA concentrations. When leaves were treated with phenidone to inhibit endogenous JA synthesis, the response in EFN production to artificial damage was much less pronounced. We observed increased numbers of defending insects and decreased numbers of herbivores on leaves after inducing EFN production by exogenous JA treatment. Over six weeks, repeatedly applied JA or artificial damage resulted in a ten-fold reduction in herbivory. Taken together, these results clearly demonstrate that EFN production can be induced by herbivory and that an induction of EFN production indeed can lead to a better indirect protection of the plants under field conditions. EFN thus represents an alternative mechanism for induced, indirect defensive plant responses that are mediated via the octadecanoid signal transduction cascade.

Key words: ant-plant interaction, jasmonic acid, mutualism, octadecanoid pathway, tropics

Mechanisms of induction of resistance to diseases in tomato plants by *Penicillium oxalicum*

Antonieta De Cal, Raúl García-Lepe, Paloma Melgarejo 183

Abstract: *Penicillium oxalicum* induces resistance against *Fusarium oxysporum* f. sp. *lycopersici*, a causal agent of tomato wilt. This resistance was accomplished with a reduction in severity in growth chamber, glasshouse and field experiments. Tomato plants were induced with a conidial suspension (10^7 conidia per ml) of *P. oxalicum* in seedbeds, seven days before transplanting. Mechanisms of induction of resistance to diseases were studied in induced and non-induced plants. No changes in protein patterns and no accumulation in the pathogenesis-related proteins (PRs) in tomato plants treated with *P. oxalicum* were observed. Also, phytoalexins were not accumulated in induced- plants. Treatment with *P. oxalicum* limited the secondary distribution of *F. oxysporum* f. sp. *lycopersici* within the vascular tissues, and prevented the distortion of cambial cells and the total differentiation of cambium caused by the pathogen. The relationship between the morphological changes related with vascular tissues induced by *P. oxalicum* in tomato plants infected with *F. oxysporum* f. sp. *lycopersici* and the production of phytohormones was investigated. Kinetin and gibberellic acid induced histological changes in vascular system of tomato plant similar to those observed in *P. oxalicum*-treated plants. Endogenous

levels of gibberellins and cytokinins were estimated in tomato plants and an increase in cytokinins (zeatin riboside or isopentenyl adenosine) was observed in plant treated with *P. oxalicum*.

Keywords: cytokinins, gibberellins, *Fusarium oxysporum* f. sp. *lycopersici*, isopentenyl adenosine, phytoalexins, rishitin, tomatine, zeatin riboside.

Characterization of induced *PR-1* in a late blight resistant *Solanum phureja*

Céline Schweitzer, Danièle Evers, Jean-François Hausman 187

Abstract: The native South-American potato species *Solanum phureja* seems to be a valuable source of field resistance against potato late blight caused by the fungus *Phytophthora infestans*. Among pathogenesis-related (PR) proteins associated with the development of an induced resistance against a broad range of pathogens, *PR-1* is an interesting candidate gene for improving resistance to oomycete diseases. The objective of this work is to characterize this *PR-1* gene in a late blight resistant *S. phureja*, including cloning and sequencing, and testing its *in vitro* activity against *P. infestans*. The final aim is the addition of *PR-1* to transgenic constructs together with other defense genes in potato.

The presence of the *PR-1* gene in resistant and susceptible parents and hybrids was shown by Southern blot. *PR-1* mRNA was induced after infection by *P. infestans*. A cDNA library was constructed and successfully screened. Cloning and sequencing of the *S. phureja PR-1* gene showed a good homology with *S. tuberosum PR-1*.

Key words: Pathogenesis-related protein, *PR-1*, late blight, potato, *Solanum phureja*, *Phytophthora infestans*.

Does Milsana[®] bioprotectant induce resistance in greenhouse as well as in field-grown plants?

Hans von Amsberg, Shunnosuke Watanabe 193

Abstract: Since the discovery of the effectiveness of extracts from *Reynoutria sachalinensis* in disease resistance induction in the 1980s by the researchers of the Biologische Bundesanstalt in Darmstadt, Germany, extensive research has been conducted in many countries. This research was mostly conducted with ethanolic extracts of REYSA, which then were formulated with additional ingredients. The formulated ethanol extracts were then tested as a suspension concentrate to verify effectiveness of induction and potential phytotoxicity.

Summary data are presented in a tabular format to show which crops and/or diseases responded to the resistance induction process. The importance of the formulation for biological unit activity and the cause of plant injury are reported in a separate experiment.

Key words: REYSA extracts, crops, diseases