

MEETING

ABSTRACTS OF PAPERS PRESENTED AT A WORKSHOP ON ECOLOGY OF *BOTRYTIS* AND *SCLEROTINIA* AND THEIR INTERACTION WITH OTHER MICROORGANISMS

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The Role of Cell-Wall-Degrading Enzymes in Virulence of *Botrytis cinerea*

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Botrytis cinerea is a fungus that can infect over 200 plants, resulting in devastating pre- and postharvest diseases in many crops. At all stages of the infection process the fungus secretes pectinolytic enzymes which have been suggested to be involved in the penetration and the invasion of plant tissue. The aim of our research is to study the role of these enzymes in pathogenesis by a molecular-genetic approach. We cloned and characterized six members of the endo-polygalacturonase (endoPG) gene family as well as an endo-pectin lyase. Phylogenetic analysis indicated that the *B. cinerea* endo-PG genes could be assigned to three distinct monophyletic groups. Expression studies, performed in several hosts at 20°C and/or 4°C, have shown a differential expression of the isolated genes. One of the endo-PG genes, *Bcpg1*, shows a basal expression *in planta* as well as in liquid culture. Elimination of *Bcpg1* by gene replacement resulted in a strain that shows a significantly reduced virulence, as well as a reduced induction of the remaining endo-PG activities. We conclude that *Bcpg1* is required for full virulence of *B. cinerea* and we postulate that the activity of the gene product BcPG1 releases oligo-galacturonides that induce expression of (some) other endo-PGs. We are currently studying the expression of the endo-PG gene family in the wild type and the *Bcpg1* null mutant by Northern blot, quantitative RT-PCR (reverse transcriptase-polymerase chain reaction) and IEF (iso-electric focusing) zymogram analysis.

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Detoxification of Preformed Plant Defense Compounds (Saponins) by *Botrytis cinerea*

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Botrytis cinerea, the causal agent of gray mold diseases of many important crops, had been reported to degrade the tomato saponin α -tomatine. Since detoxification of saponins has been shown to be an important factor determining host specificity in other systems, we investigated the role of saponin degradation for the colonization of tomato and other host plants by *B. cinerea*. We show that *B. cinerea* deglycosylates not only α -tomatine, but also several other saponins (avenacin,

avenacosides, digitonin) and that at least three different enzymatic activities are involved. The detoxification of α -tomatine is achieved by removal of the terminal xylose, *i.e.*, the *B. cinerea* tomatinase is a xylosidase. A strain lacking tomatinase activity (M3) is highly sensitive against α -tomatine and does not infect tomato plants (but, *e.g.* beans), indicating an important role of this tomatin detoxification for the colonization of tomato. M3 also lacks the ability to deglycosylate digitonin, which, too, involves removal of a xylose moiety. Using the *S. lycopersici* tomatinase gene as a probe, we cloned a gene, *sap1*, which shows significant homology to the probe and to the avenacinase gene of *Gaeumannomyces graminis*. *Sap1* deletion mutants obtained by a gene replacement approach lost the ability to degrade avenacin, *i.e.*, *sap1* obviously codes for an avenacinase-like enzyme; *sap1* strains can still degrade avenacosides. In summary, *B. cinerea* produces at least three enzymes involved in saponin degradation: a xylosidase (α -tomatine, digitonin) and two glucosidases (avenacin/avenacosides).

Integrated Cultural, Environmental and Biological Control of Gray Mold (*Botrytis cinerea*) in Greenhouse Crops

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Gray mold (*Botrytis cinerea*) can seriously affect production of greenhouse salad crops in the UK. Increasingly, growers and supermarkets agree on production codes of practice which place restrictions on fungicide use in addition to state legislation. Crop and environment management are thus important strategies for disease control, while there is renewed interest in biological control. *Botrytis* frequently establishes initially on senescent tissue. In cucumber and tomato, older leaves are regularly removed to prevent stem infection by this route; old fruit trusses are also removed in some tomato crops. Recent experiments on tomato demonstrated a benefit in not leaving a stub at leaf or truss removal. In lettuce, optimization of soil and plant conditions to ensure rapid rooting and to prevent leaf yellowing and wilting, can reduce occurrence of *Botrytis* basal rot. In all three crops – cucumber, tomato and lettuce – there is evidence that plant nutrition can affect susceptibility to *Botrytis*. In long-season tomato and in autumn cucumber crops, maintenance of a high minimum temperature in the hot water pipes reduced *Botrytis* stem rotting. Where tomato crops are layered, laying the horizontal stems on hooped supports – to keep them off the wet floor – reduced stem *Botrytis*. In Israel, biological control of *B. cinerea* on cucumber and tomato was demonstrated using *Trichoderma harzianum* T39 (Trichodex). In England, recent studies have identified isolates of *Bacillus subtilis* capable of controlling *B. cinerea* on lettuce. Variability in control efficacy and the high cost of registration have delayed widespread adoption of biological control. Although cultural practices, environment management and use of biological control agents may each provide only partial control of gray mold, integration of these practices increases the probability of achieving reliable, acceptable control.

***Pseudomonas aeruginosa* TNSK2 and *Trichoderma harzianum* T39 Induce Resistance to *Botrytis cinerea* on Bean and Tomato**

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After a local attack with a necrotizing pathogen, plants can develop a long-lasting systemic resistance (SAR) towards subsequent attacks from the same or other pathogens. SAR is accompanied by a local and systemic increase in endogenous salicylic acid levels. Also root or seed inoculation with some nonpathogenic microorganisms can induce systemic resistance, called ISR since, unlike

SAR, it can be salicylic acid-independent. In the present study, it was investigated whether SAR/ISR also works against necrotrophic pathogens such as *Botrytis cinerea*. Leaf infections with 10^6 *B. cinerea* spores/ml were carried out in model systems using bean or tomato plants. Both *Pseudomonas aeruginosa* 7NSK2 and *Trichoderma harzianum* T39 induced systemic resistance against *B. cinerea* on bean and tomato and stopped spread of the pathogen at a very early stage. When the infection pressure was very high, however, *B. cinerea* spread could not be controlled effectively by induced resistance. Salicylic acid-negative mutants of *P. aeruginosa* 7NSK2 no longer induced ISR. Moreover, resistance to *B. cinerea* could be induced by applying nano-molar concentrations of salicylic acid or BTH (Bion, a salicylic acid analogue) to the roots of bean plants. By using tomato plants affected in various signaling pathways (never ripe, sitiens and NahG plants), we are currently studying which pathways are activated by *P. aeruginosa* 7NSK2 and *T. harzianum* T39. Also the mechanism(s) by which *T. harzianum* T39 induces resistance against *B. cinerea* are under investigation.

Biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* with *Trichoderma* spp. and *Coniothyrium minitans*

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Investigations have been performed in multiannual field trials (1991–1997) with the micromyceta *Trichoderma harzianum*, *T. viride*, *Coniothyrium minitans* and *Saccharomyces chevalieri* and similar bioproducts of these fungi (Trichodex 25WP – *T. harzianum* T39, Makhteshim Agan, Israel; Trichosenim 25PTS, Trichopulvin 25PU, *Trichoderma* pellets, *T. viride*, isolate Td 50; Saccharopulvin 25 PU, *S. chevalieri*, RIPP, Bucharest, Romania) to protect grapevine against gray mold (*Botrytis cinerea*) and sunflower, soybean and beans against white rot (*Sclerotinia sclerotiorum*). Sprays with Trichodex in seven vineyard locations were efficient in significantly decreasing gray mold attack, when applied alternately with chemicals, as follows: treatments 1 (after flowering) and 3 (at start of grapevine ripening), using an anti-*Botrytis* fungicide: Sumislex 50 WP (0.1%) / Ronilan 50 WP (0.1%) / Rovral 50 WP (0.1%) / Konker SC (0.1%) and treatments 2 (at grape bunch formation) and 4 (3 weeks before harvest) with Trichodex (0.2%) at 1,000 l/ha. The last Trichodex application ensured a high quality harvest, without residues, fit for table grapes and winemaking. All biological seed or soil treatments (*Trichoderma*, *Coniothyrium*) showed good efficacy in protecting sunflower, soybean and beans from white rot (*S. sclerotiorum*), but lower than that of the specific standard chemicals (Sumislex 50 WP, Metoben 70 PP, Tiramet 60 PTS). Seed yields harvested in biological treatment trials were higher than those obtained from the untreated check. Although the efficacy of biological products was lower than that of chemicals, the former are advantageous in protecting the agroecosystems of sunflower, soybean and bean and the environment, as well.

Mode of Action of *Trichoderma harzianum* T39: Competition, Control of Pathogen Pathogenicity Enzymes and Induced Resistance

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Biological control of gray mold (*Botrytis cinerea*) and of white mold (*Sclerotinia sclerotiorum*) can be achieved by *Trichoderma harzianum* T39. This biocontrol agent is used for the control of *B. cinerea* on grapes, for the control of both diseases on greenhouse crops, as well as for the control of other diseases. The biocontrol mechanism by which *T. harzianum* T39 is active was investigated. *T. harzianum* T39 inhibits the conidial germination and germ tube elongation of *B. cinerea* by competition for sources such as nutrients and space that are in short supply for the pathogen. Inhibitory compounds (antibiosis) or fungal cell wall-degrading enzymes (mycoparasitism) are not involved in the biocontrol process. Although after one day it is expected that the germ-tubes would penetrate the host tissue, penetration is also suppressed. This indicates another possible mechanism. In the presence of *T. harzianum* T39, the activity of the hydrolytic enzymes cutin esterase, exo- and endo-polygalacturonase, pectin methyl esterase and pectate lyase on *B. cinerea* infected bean leaves is reduced. Carboxymethyl cellulase was not affected by *T. harzianum* T39. A serine protease currently isolated and characterized, which originates from T39, was isolated and found to be responsible for restraining the pathogen's pathogenicity. The fact that dead cells of *T. harzianum* T39 applied to the leaves or live cells to the root zone of plants resulted in the reduction of foliar diseases, points to the involvement of induced resistance.

Hypovirulence Associated with Double-stranded RNA in *Sclerotinia* spp.

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The biology and potential utility of hypovirulent phenotypes associated with double-stranded RNA (dsRNA) in *Sclerotinia* spp. are being investigated. Hypovirulent phenotypes in *Sclerotinia sclerotiorum*, *S. minor* and *S. homoeocarpa* have been associated with dsRNA ranging in size from ca 2 to 12 kb, and dsRNA and hypovirulent phenotypes were intra-specifically transmitted between selected isolates. In *S. sclerotiorum*, dsRNA was not associated with viral particles but with double membrane-bound bodies within cells of mycelia and sclerotia. Selected hypovirulent and virulent isolates were assessed in biological control experiments in growth room and field trials. In *S. minor*, foliar sprays of mycelial suspensions of hypovirulent isolates onto expanding lesions on lettuce reduced or halted lesion expansion and sclerotial production when isolates were mycelially compatible but not when they were incompatible. In *S. homoeocarpa*, applications of a hypovirulent isolate to diseased turf suppressed disease in growth room and field trials, and disease suppression in field trials persisted into the following year. In host range studies, a hypovirulent phenotype and dsRNA from *S. sclerotiorum* were interspecifically transferred into *S. minor* through hyphal anastomosis. The recipient isolate of *S. minor* developed the hypovirulent phenotype and contained dsRNA from the donor isolate of *S. sclerotiorum*. The results confirm that dsRNA-associated hypovirulence is present among isolates of *Sclerotinia* spp. and that, under defined conditions, such isolates can provide effective disease suppression. Molecular characterization of selected dsRNAs from *Sclerotinia* spp. has been initiated.