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Antagonists screening

Using community and species diversity data to discover novel biocontrol bacteria

Brian B. McSpadden Gardener 3-6

Abstract: Discovery of new bacterial biocontrol agents will be essential for the expansion of biopesticidal product lines. Identifying and recovering microbes which consistently act to suppress plant diseases is a necessary first step in the development and application of new active ingredients. Historically, the approach has been to recover microbes and then screen them for activity in various contexts. While this approach to “recover then identify” biocontrol agents has been successful, most of the recovered isolates that remain in the final screens belong to a limited set of genera. To overcome this limitation and expand our view of the diversity of bacterial biocontrol agents present in nature, we have developed and applied two molecular approaches to first “identify then recover” important biocontrol bacteria. These two approaches have resulted in the rapid recovery of novel and effective strains of diverse biocontrol bacteria.

Can mycoviruses be used for the biocontrol of the plant pathogenic fungus *Botrytis cinerea*

Michael Pearson, Ross Beever, Barbara Boine, Colin Tan 7-10

Abstract: The necrotrophic plant pathogen *Botrytis cinerea* is a major horticultural pathogen worldwide. Control methods for this pathogen rely heavily on fungicides with their associated problems of resistance and chemical residues. Biocontrol offers an alternative approach and we are exploring the use of mycoviruses for this purpose. For successful exploitation of mycoviruses in this way, they must have some deleterious effect against the target fungus and be able to spread and infect fungal populations in the field. We have sequenced two filamentous ssRNA viruses (BVX and BCVF) from *B. cinerea* and are assessing their effects on fungal fitness and pathogenicity. Viral transmission in the field is presumed to occur mainly by hyphal fusion and vegetative incompatibility will likely limit transmission by this route in *B. cinerea*, which has >66 vegetative compatibility groups. We have detected BVX and BCVF in *B. cinerea* isolates from several countries and have demonstrated that BVX is transmitted both through asexually produced conidia and sexually produced ascospores. It may prove feasible to use these viruses as gene vectors and produce infectious clones to increase their virulence and transmissibility.

Endophytes as biological control agents for plant pathogens

David Ezra, Tamir Lousky, Yigal Elad 11-14

Abstract: Microorganisms have always been a source of secondary metabolites used for the benefit of human kind. Many of the antibiotics used against pathogens originate from bacteria and fungi. Other metabolites produced by microorganisms are being used in the industry. Using microorganisms and their products for the control of pathogens in agriculture is not new. Some bacterial and fungal biocontrol agents (BCAs) or their secondary metabolites are registered for the control of plant disease in organic or conventional farming. The use of endophytes as BCAs and as the source of secondary metabolites is not common yet. We have been looking for endophytes in trees with the aim of discovering those with antibiotic activity able to affect tree pathogens and especially systemic ones. The involvement of woody tissue, large plants and long life cycle makes it difficult to suppress systemic fungal diseases, by using chemical and cultural methods. The idea of combining the endophytes ability to develop systemically in trees, to

produce and secrete biologically active secondary metabolites and to suppress systemic pathogens of trees is a logical approach. Recognizing and characterizing those secondary metabolites will enable us to exploit and use it in large scale for the control of plant pathogens in fruit trees. In the last few years, we have isolated and tested over a thousand endophytes from different trees in Israel, many of them with biological activity against plant pathogens. Some were found to secrete secondary metabolites with a wide range of inhibition against a diversity of pathogens while others were specific to certain pathogens. A very unique endophyte that we have isolated was found to emit active volatiles that may be used in the control of pathogens in postharvest and storage of fruit, vegetables and seeds. Molecular identification of active endophytic fungi by rDNA 5.8S ITS region and actin and β -tubulin gene sequencing was performed.

Screening of microorganisms with potential activity against *Plasmopora viticola*

Silvia Dagostin, Davide Gobbin, Tiziano Formolo, Ilaria Pertot 15-18

Abstract: Downy mildew, caused by the obligate oomycete *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni., is one of the most important grapevine diseases occurring worldwide. No effective alternatives to chemical fungicide are currently available. The interest in the use of biocontrol agents (BCAs) is increasing, but mass screening for biocontrol efficacy on plants is expensive and time consuming. Therefore, a rapid *in vitro* method for high-throughput screening was developed. The method is based on leaf disk assay and relative quantitative real-time polymerase chain reaction (qRT-PCR). Leaf disks were cut from susceptible *Vitis vinifera*, kept in contact with suspensions of cells of each microorganism and then inoculated with *P. viticola* sporangia. After incubation, DNA was extracted and analysed with real-time PCR. Ratio of CT *V. vinifera* over CT *P. viticola* (infection coefficient IC) was used as biocontrol activity indicator. A high ratio (IC~1) indicated a successful infection and tissue colonization by *P. viticola* and a poor control activity, conversely, a low IC (IC~0.5) indicated a good disease control. The validation of the method was carried out by assays on plants. Seven microorganisms were chosen. Under greenhouse controlled condition, grapevine plants were treated with selected microorganisms and then infected with *P. viticola*. After the incubation period, samples were collected from plants and analysed with qRT-PCR method. Subsequently, after sporulation, severity (percentage of infected leaf) of disease was assessed. PCR results from *in vitro* and greenhouse experiments were compared to severity on plants. Pros and cons of this method are discussed.

Characterisation of microorganisms isolated from summer-wheat and testing for their potential inhibitory effects on *Fusarium graminearum*, causal agent of Fusarium Head Blight (FHB) in laboratory screening-tests

Birgit Antlinger, Sabine Frühauf, Marc Lemmens, Rudolf Braun, Markus

Neureiter 19-22

Abstract: Potentially antagonistic microorganisms were isolated from two cultivars of wheat, differing in their susceptibility to Fusarium head blight caused by *Fusarium graminearum*, at various growth stages and from different tissues of the plant surface. Isolates were characterised by RAPD-PCR in order to exclude identical strains and to obtain information about the phylogenetic distance in between them by analyzing their fingerprints. Furthermore a screening in a dual-culture plate test was performed to investigate their impact on the behavior of the plant pathogen. A second assay was established to examine the possible secretion of secondary metabolites, which potentially inhibit conidia germination, respectively growth of the fungus.

Nicotinic acid degradation: a novel method for selection of a biocontrol agent against

Erwinia amylovora

Thomas Paternoster, Brion Duffy, Geneviève Défago 23-25

Abstract: *Erwinia amylovora* is the causal agent of fire blight, often devastating disease of apple, pear, and other rosaceous plants. The disease is usually initiated by epiphytic populations of *E. amylovora* developing on blossoms. The pathogen requires nicotinic acid and thiamine as growth factors in laboratory culture media. Unlike thiamine, which is required by a few wild-type strains, nicotinic acid is a specific requirement among species of the genera *Erwinia*. Starting from these evidences, the aim of the work was to select a new biocontrol agent against fire blight making use of this specific pathogen's peculiarity. For this purpose, a collection of 735 bacteria and 1237 epiphytic yeast, respectively, was created from apple and pear flowers collected in orchards situated in different locations of Switzerland and Trentino. The whole collection was screened for the ability to degrade nicotinic acid in a system based on the use of nicotinic acid as sole N-

source. Ten percent of the isolates showed this capacity. Among several isolates tested, JAN strain displayed the best growth performance and the strongest biocontrol effect against *E. amylovora* in pear slice bioassay. JAN1 F12 strain was characterized as *Pseudomonas rhizosphaerae* species by 16S rDNA gene sequence analysis. The strain proved to be a competent colonizer of apple blossoms, and strongly suppressed pathogen growth in detached flower assays and in greenhouse flowering apple tree trials with reduced development of blossom blight.

Biological control of walnut blight: screening of antagonistic bacteria for *Xanthomonas arboricola* pv. *juglandis* and evaluation of their efficacy

Hatice Ozaktan, Mine Erdal, Ahmet Akkopru, Adem Bozkurt, Emek Aslan 26-29

Abstract: Bacterial blight of walnut, caused by *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) is present in all main areas of walnut production in the world, although the degree of economic importance is directly related to the climate. Severe crop losses have been observed when frost injury and rains followed by warm temperatures coincide with the budbreak period in the early spring of the previous year in the Western part of Turkey. Copper-based compounds have been the only means of control for more than 40 years. Data indicates that copper-resistant strains of the walnut blight pathogen are not killed by standard copper applications under field conditions. Biological control of walnut blight pathogen may be alternative to chemical control. The goal of this study was to determine whether bacterial antagonists could be used to control of *Xaj*, the causal agent of bacterial blight on walnut. In total, 35 bacterial antagonists, of which 29 bacterial strains were isolated as epiphytes from phylloplane of healthy walnut trees, were screened for their *in vitro* biocontrol activity to *Xaj*. *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A506 were also tested as reference biocontrol agents against *Xaj*. Approximately 52% were identified as fluorescent pseudomonads, 28% Gram positive bacteria, and 20% Gram negative bacteria. Of the 35 FP strains tested, 18 inhibiting the growth of *Xaj* between 3.0 mm to 13.0 mm on TSA plate were selected and tested for their ability to control of bacterial blight of walnut on the immature nut test. Approximately 39% of the antagonistic bacterial strains tested on immature nut test, significantly reduced bacterial blight of walnut by 73 to 88% compared to the pathogen-alone treatment. Our research will continue with *in vivo* efficacy tests on walnut seedlings.

***Lysobacter* spp. as potential inhibitor of *Rhizoctonia solani* in soil (Abstract)**

Barbara Drigo, Mirjam Schilder, Joeke Postma 30

Abstract only

Screening of actinomycetes from medicinal plant rhizosphere soils for antifungal compounds, indole-3-acetic acid and siderophores production (Abstract)

Sutthinan Khamna, Akira Yokota, Saisamorn Lumyong 31

Abstract only

Selection of *Pseudomonas* strains that are antagonistic to *Verticillium fungicola*, the causal agent of dry bubble disease in *Agaricus bisporus* (Abstract)

Roeland L. Berendsen, Peter A.H.M. Bakker 32

Abstract only

Molecular/biochemical characterisation and monitoring

Genotypic comparison of *Pantoea agglomerans* biocontrol and clinical isolates to address taxonomic and bio-safety questions

Fabio Rezzonico, Theo H.M. Smits, Cosima Pelludat, Emilio Montesinos, Jürg E.

Frey, Brion Duffy 35-39

Abstract: The species *Pantoea agglomerans* includes very promising biocontrol organisms not only against fire blight on apple and pears, but also against other bacterial and fungal diseases. However, *P. agglomerans* is currently listed as a biosafety level 2 (BL2) organism due to clinical isolates reported as opportunistic human pathogens. The aim of this work was to assess the biosafety of *P. agglomerans* strains and to search for markers enabling the selection of safe biocontrol strains. Multi-locus phylogenetic analysis, fluorescent Amplified Fragment Length Polymorphisms (fAFLP) fingerprinting and comparative genomics were employed, together with the scrutiny of clinical data, to characterize a collection of environmental, biocontrol and clinical isolates.

Molecular characterization of *Ampelomyces* spp. isolates from different hosts and geographic origins and evaluation of their potential to control powdery mildew of cucumber

Dario Angeli, Elisabetta Pellegrini, Monika Maurhofer, Ilaria Pertot, Susanna Micheli, Denise Röss, Cesare Gessler 40-44

Abstract: *Ampelomyces quisqualis* is a widespread mycoparasite of *Erysiphales*. The phylogenetic relationships of twenty *Ampelomyces* spp. isolates were inferred from the sequence of their ITS rDNA. The *A. quisqualis* strains were obtained from culture collections or isolated from *E. necator* in northern Italy (Trentino-Alto Adige region). Based on the phylogenetic ITS analysis the isolates were clustered into three different genetic groups. The five isolates from Trentino-A.A. region grouped into two distinct groups. Three Trentino isolates were similar to some isolates from North America and the other two isolates formed a distinct group with strains isolated from different countries and host plants. The biocontrol efficacy of all *A. quisqualis* isolates against cucumber powdery mildew (*Podosphaera xanthii*), measured as reduction of powdery mildew conidia production, greatly varied between the isolates. However, no correlation between the phylogenetic groups and aggressiveness against cucumber powdery mildew was found.

Antifungal peptides of the biocontrol fungus *Trichoderma atroviride* – detection and structure characterisation by LC/MS/MS

Rainer Schuhmacher, Norbert Stoppacher, Barbara Reithner, Markus Omann, Susanne Zeilinger, Rudolf Krška 45-48

Abstract: Biocontrol strains of *Trichoderma* spp. are filamentous soil fungi that are able to parasitise/parasitize plant pathogenic fungi such as *Botrytis cinerea* and induce defense mechanisms in plants. For both processes, secondary metabolites of *Trichoderma* (e.g. peptaibiotics) have been shown to play a key role. Peptaibiotics are non-ribosomal linear peptides containing unusual amino acids like alpha-aminoisobutyric acid (Aib) and show antibiotic properties. In this work, we have screened and characterised peptaibiotics in fungal culture samples of *Trichoderma atroviride* ATCC 74058 (*T. atroviride* P1) using liquid chromatography/tandem mass spectrometry (LC/MS/MS). After enrichment of the antifungal peptides by solid-phase extraction (SPE) and their subsequent chromatographic separation, they were identified and characterised by various MS and MS/MS techniques. We have found 20 Aib-peptides of the trichorzianine group in the fungal culture samples of *Trichoderma atroviride* ATCC 74058. In subsequent analyses, another 15 peptaibiotics of 7-9 amino acids length were detected for which we suggested the group name “Trichoatrokontins”.

Molecular tools for studying the interaction between *Botrytis* and the viruses BVX and BVF (NZ)

Barbara Boine, Mike N. Pearson, Ross Beever, Andy Bailey, Gary Foster 49-52

Abstract: Understanding the nature of the relationship between viruses and their fungal hosts is critical in determining the ecological significance of mycoviruses and their potential usage as biological control agents. Two flexuous viruses belonging to the family Flexiviridae, *Botrytis cinerea* virus F (BCVF) and *Botrytis* virus X (BVX), from *Botrytis cinerea* have previously been completely sequenced, providing the opportunity to examine their interaction with *B. cinerea* at molecular level. In addition, studying their impact on the virulence of *Botrytis* is also of great interest. In order to study the fungus/virus interaction four basic tools were developed: i) an efficient transfection protocol to introduce viruses into uninfected fungal isolates (ii) a transformation protocol to incorporate plasmid DNA into *Botrytis*, iii) a consistent and reliable real-time PCR detection method for BCVF and BVX to study the effect of virus transfections, and iv) an immunoassay for BVX to visualize the virus distribution and movement within the mycelia and also between compatible fungal strains. These tools will enable the study of the relationship between the fungus and the mycoviruses at the cellular level.

Quantitative strain specific molecular methods for monitoring and ecological fitness studies of biological control agents

Emilio Montesinos, Marta Pujol, Esther Badosa 53-57

Abstract: Specific analysis of biocontrol agents at strain level is necessary for field studies about improvement of formulation, ecological fitness, traceability, residue analysis, and environmental impact. A quantitative strain specific method have been developed for the biocontrol agent of fire blight, *Pseudomonas fluorescens* EPS62e. The comparison of patterns of RAPDs and U-PCR within a wide collection of representative strains of the same species identified specific marker gene sequences of EPS62e which were used to design primers for real-time PCR (quantitative PCR). The combined use of rtPCR and culture-based methods of analysis of strain EPS62e, in apple and pear orchards, in France and Spain, identified three physiological states in the field, which consisted of active colonization, survival and entry into a viable but nonculturable state, and cell death. It was concluded that the combination of quantitative molecular and culture-based methods is a powerful tool for monitoring biocontrol agents because provides information about its environmental fate.

Molecular and functional characterization of induced systemic resistance in grape

Michele Perazzolli, Elisa Bozza, Claudio Moser, Yigal Elad, Iliaria Pertot..... 58-61

Abstract: Strains of non-pathogenic microorganisms can reduce plant diseases through activation of a plant-mediated defence mechanism known as induced systemic resistance (ISR). Scarce knowledge is available on the mechanisms of activation of ISR. Our aim was to characterize the systemic resistance against downy mildew activated in grapevine by the biocontrol agent *Trichoderma harzianum* T39. *T. harzianum* T39 reduced symptoms severity similarly to a benzothiadiazole (BTH) treatment. Optimal disease control was obtained when *T. harzianum* T39 was applied more than once before pathogen inoculation, whereas a single application of BTH was sufficient. In both cases a stronger effect was observed on leaves directly treated with the agents, but an efficient systemic protection was observed as well on untreated leaves. Expression analysis revealed the absence of *PR2* and *PR4* induction after elicitation and absence of priming after pathogen challenge.

Molecular and biochemical characterization of Iranian surfactin producing *Bacillus subtilis* isolates

Salehi Jouzani Gholamreza, Matin Mohamadipour, Maryam Mousivand, Saeed Abbasalizadeh, Mohammad Salari, Naser Panjeh keh 62-66

Abstract: Characterization of surfactin-producing *Bacillus subtilis* isolates collected from different ecological zones of Iran was performed using blood agar, PCR, drop collapse assays and reverse-phase high performance liquid chromatography (HPLC), and their biocontrol effect against *Aspergillus flavus* and *Colletotrichum gleosporioides* was studied. Totally, 290 *B. subtilis* isolates were isolated from phyllosphere and rhizosphere samples collected from fields and gardens of five provinces of Iran. Isolates containing *sfp* gene, encoding surfactin, were detected using the PCR method. It was found that 14 different isolates contained the *sfp* gene. Drop collapse assays which detect isolates with high production of surfactin showed that seven isolates produce high levels of surfactin. It was found from HPLC analysis that the isolates containing the *sfp* gene produce between 55 and 1610 mg surfactin per liter broth medium. Four isolates, including BS119m, BS116l, N3dn and BS113c produced more than 1000 mg surfactin per liter of broth. The highest surfactin production level was observed for isolate BS119m (1610 mg/l). In bioassays isolate BS119m exhibited strong inhibitory effects against *A. flavus* (100%) and *C. gleosporioides* (88%). Furthermore, the effect of purified surfactin on growth of *A. flavus* was evaluated. Mycelium growth was considerably reduced with increasing concentrations of surfactin and 36, 54, 84 and 100% inhibition of mycelial growth, respectively, was observed at 20, 40, 80 and 160 mg/l after seven days of incubation.

Molecular characterization of IAA-responsive mutants of plant-beneficial *Pseudomonas fluorescens* CHA0 (Abstract)

Laurene Rochat, Maria Péchy-Tarr, P. de Werra, Monica Maurhofer, Christoph Keel 67

Abstract only

Enhancement of efficacy

Development of a genetically-modified mixture of biological control agents for improved disease control

Virginia O. Stockwell, L. Meadow Anderson, Joyce E. Loper, Kenneth B.

Johnson 71-74

Abstract: Adoption of bacterial antagonists for disease management is hampered by inconsistent performance. Intergeneric mixtures of antagonists may reduce variation in control by establishing a robust community on plant surfaces and greater competition to the pathogen during its critical epiphytic growth stage. *Pseudomonas fluorescens* A506 and *Pantoea agglomerans* Eh252, bacterial antagonists for the management of fire blight of pome fruit trees, are examples of ecologically-compatible bacterial antagonists. A506 suppresses colonization by the pathogen by competitive exclusion. Strains of *P. agglomerans*, such as Eh252, suppress pathogen growth by competition and production of an important small peptide antibiotic. When applied in a 1:1 mixture, A506 and Eh252 often established greater population sizes on plant tissues; however, disease control was not significantly improved by the mixture compared to single strains. In laboratory assays, we found that A506 produced an extracellular metalloprotease that detoxified the peptide antibiotic of Eh252. We attribute the lack of synergism of the two strains in disease control to ‘mechanistic incompatibility’ or the interference in antibiosis by Eh252 by the protease of A506. We selected a Tn5 mutant of A506 deficient in production of the extracellular protease. The protease-deficient mutant colonized plant tissues and provided similar levels of disease control as wild-type A506. Combining the protease-deficient derivative of A506 with Eh252 increased disease control to levels that are significantly greater than single strain inoculants. Altering A506 to be mechanistically compatible with its co-inoculant Eh252 improved disease suppression and decreased variability in biological control of fire blight.

Introduction of the endochitinase ChiA gene from *Serratia plymuthica* enhances biocontrol activity of 2,4-DAPG-producing strains of *Pseudomonas fluorescens*

Inbal Gazit-Fatal, Marianna Ovadis, Rena Gorovits, Ada Viterbo, Ilan Chet,

Linda Thomashow, Leonid Chernin 75-78

Abstract: The 1.6-kb gene *chiA* encoding the 58-kDa endochitinase ChiA from *Serratia plymuthica* IC1270 was cloned into the integrative pBK-miniTn7-ΩGm plasmid under control of the *tac* promoter. The construct was employed to enhance antifungal activity and broaden the range of target pathogens of *Pseudomonas fluorescens* strains Q8r1-96 and Q2-87. Both strains produce antibiotic, 2,4-diacetylphloroglucinol (DAPG), responsible for biocontrol of *Gaeumannomyces graminis* var. *tritici* (*Ggt*), but relatively inefficient against some other plant pathogens, including *Rhizoctonia solani*. Recombinant derivatives of Q8r1-96 and Q2-87 carrying the *tac-chiA* cassette inserted into the chromosome acquired chitinolytic activity. Introduction of *tac-chiA* cassette increased the ability of the wild type strains to protect beans against *R. solani* in greenhouse. Moreover, the DAPG-minus mutant Q2-87DZ carrying *tac-chiA* suppressed *R. solani* similarly to the parental strain, indicating that acquired chitinase activity may substitute for a deficiency in DAPG production.

Molecular approaches to improve biocontrol of soil-borne fungal pathogens

Gabriele Berg, Henry Müller, Christin Zachow, Rita Grosch, Leo Eberl, Wolfgang

Vogt, Ralf Tilcher 79-82

Abstract: The highly toxic and climatically relevant soil fumigant methyl bromide was used to suppress soil-borne pathogens, which cause high yield losses world-wide. To develop environmentally friendly alternatives in plant protection, two target pathogens with a broad host range and high economic importance were studied: *Verticillium dahliae* Kleb. and *Rhizoctonia solani* Kühn. Strategies to select the most efficient antagonists were developed on the basis of hierarchical systems combining microbiological, molecular and phytopathological methods. To control *Verticillium* wilt, the biocontrol agent (BCA) *Serratia plymuthica* HRO-C48 was selected. The strain shows high antifungal and plant growth promoting activity and poses no risk for the environment and human health. RhizoStar® is registered and produced by E-nema, Raisdorf, Germany. For biocontrol of *Rhizoctonia* diseases another strategy was developed: a combination of fungal and bacterial antagonists has been applied. While the fungi are used for direct disease protection and parasitism of sclerotia and hyphae, endophytic bacteria are applied

to enhance plant growth and to protect against the pathogen from outside and inside the rhizosphere. The mode of action of BCAs and of interaction with microbial communities was studied using molecular and microscopic techniques (FISH in combination with Confocal Laser Scanning Microscopy). Results of these investigations lead to new strategies in application and formulation of BCAs.

The *Trichoderma*–plant–pathogen interaction: understanding the mechanism and improving biocontrol

Sheridan L. Woo, Michelina Ruocco, Francesco Vinale, David Turrà, Roberta Marra, S. Lanzuise, Khaled Abadi, Matteo Lorito 83-88

Abstract: Our current understanding is that the activity of a fungal biocontrol agent is not limited to the direct killing or inhibition of the pathogen, but also involves extensive changes in the manner in which plants and pathogens interact. By using *Trichoderma* spp. as a model, we demonstrated that these saprophytic microbes establish a type of symbiotic relationship with the plant. They stimulate root formation, and consequently plant growth and crop yield, in order to increase the area to colonize and the quantity of exudates to use as nutrients. At the same time, effector proteins are released by the biocontrol agent within the plant tissues and “sensed” by the plant cells, resulting in defence-related reactions such as spikes in Ca²⁺ uptake and PCD. This generates a pre-activation of the systemic resistance to pathogens and a deep change in the plant “interactome” produced in response to pathogen attack. The outcome of this complex beneficial effect is that crops colonized by effective *Trichoderma* strains grow better, are more resistant to both foliar and soil-borne diseases, and are less susceptible to abiotic stresses such as lack of nutrients, drought, etc. The knowledge gathered so far allows the development of a new generation of bio-agents capable of protecting the plant from various stresses and displaying both the fertilizer and disease control effects.

Microbial mixtures enhancing plant resistance to pathogen stress

Magdalena Szczech, Beata Kowalska, Barbara Dyki, Marcin Horbowicz, Waldemar Kowalczyk 89-94

Abstract: A community of active microorganisms with diverse mechanisms of disease suppression may enhance the efficacy of biocontrol. In previous studies, the authors obtained protective effects and enhanced yield using mixtures of several bacteria (strains CAT5, PT60 and B125) and antagonistic *Trichoderma harzianum* PBG for inoculation of tomato and cucumber plants grown in potting medium infested with multiple pathogens. Plant growth promotion and induction of resistance in plants treated with the mixtures were suggested as possible mechanisms of protection. To study this hypothesis tomato and cucumber seeds were inoculated with mixtures of active bacteria and *T. harzianum* PBG or with suspensions of single microbial strains, and grown in potting medium infested with multiple pathogens. After six weeks of growth root biomass was measured. Then, microscopic studies were performed to observe the presence of defense reactions at the root level. The content of phenolic compounds in the roots was estimated. Possible induction of systemic resistance in plants treated with the microorganisms was studied on detached leaves inoculated with *Botrytis cinerea*. It was found that combinations of microorganisms enhanced root development compared to noninoculated control plants and increased the lignifications of tomato plants. Seed treatment with a mixture of strains PBG+PT60+B125 resulted also in reduction of necrosis development on leaves infested with *B. cinerea*.

Biocontrol of *Rhizoctonia solani* with improved *Trichoderma harzianum* strains in tomatoes cropped under glasshouse and commercial conditions

Jaime Montealegre, Soledad Sánchez, Fabián Ochoa Rodrigo Herrera, Ximena Besoain, Luz M. Pérez 95-98

Abstract: Biocontrol of *R. solani* was investigated using wild and improved strains of *T. harzianum* obtained by protoplast fusion, and selected and characterized for their good biocontrol activity against *R. solani*. Pellets containing different *Trichoderma* strains (1.7 g pellet/L soil) were applied to a soil inoculated with *R. solani* A- G 4 at transplant. Controls without *Trichoderma* and with added fungicide were compared with each strain used, under glasshouse conditions. Results showed that improved *Trichoderma* controlled better *R. solani* than their original wild strains. Best results, were obtained with the strains ThF2-1 and ThF5-8, when canker and mortality levels were evaluated. An experiment run with the commercial tomato crop

cv. Fortaleza, using improved strains obtained after irradiation with UV light 320 nm (Th12 A 10.1) and protoplast fusion (ThF2-1) and controls with CH₃Br and a commercial *Trichoderma* formulation, showed that the best results were obtained using ThF2-1 and the commercial formulation of *Trichoderma* spp., when applied in three opportunities. These results indicate that the improved strains are more efficient for the control of *R. solani* than their corresponding wild types in tomatoes cropped under glasshouse and commercial conditions.

Biocontrol mechanisms

Involvement of swollenin, an expansin-like protein from *Trichoderma*, in myco-parasitism

Ada Viterbo, Yariv Brotman, Eden Briff, Ilan Chet..... 101-104

Abstract: Swollenin, a protein first characterized in the saprophytic fungus *Trichoderma reesei* (Saloheimo et al., 2000), contains an N-terminal Carbohydrate Binding Module Family 1 domain (CBD) and a C-terminal expansin-like domain. This protein was identified by LC/MS among many other cellulolytic proteins secreted in the co-culture hydroponics medium of cucumber seedlings and *T. asperellum*, a well known biocontrol agent and inducer of plant defense responses. *Trichoderma* transformants overexpressing this protein showed a remarkably increased ability to colonize cucumber roots. Root colonization rates were reduced in transformants silenced in swollenin gene expression. These same transformants were capable to overgrow *Rhizoctonia solani* but not *Pythium aphanidermatum* in plate confrontations assays. Real time PCR analysis could show that the swollenin gene is up-regulated during mycoparasitic interaction with *P. aphanidermatum* but not with *R. solani*.

A cAMP receptor-like GPCR is involved in *Trichoderma atroviride* mycoparasitism

Markus Omann, Sylvia Lehner, Kurt Brunner, Marizela Delic, Norbert Stoppacher, Rainer Schuhmacher, Marion Pucher, Susanne Zeilinger..... 105-108

Abstract: G alpha \square subunits affect several processes including host recognition and activation of the mycoparasitic response in *Trichoderma atroviride*. To extend our knowledge on G protein signalling during *Trichoderma* biocontrol, we aimed to analyse G protein-coupled receptors (GPCRs). As the genome sequence of *T. atroviride* is not publicly available yet, we carried out an in silico exploration of the genome of *T. reesei*, a close relative, resulting in 20 genes encoding putative GPCRs distributed over eight classes and additional 32 proteins similar to the *Magnaporthe grisea* PTH11 receptor. Subsequently, four *T. atroviride* GPCR-encoding genes were isolated and affiliated to the cAMP receptor-like (CRL) family by phylogenetic and topological analyses. All four receptor-encoding genes showed lowest expression on glycerol and highest mRNA levels upon carbon starvation. Transcription of *gpr3* and *gpr4* responded to exogenously added cAMP and the shift from liquid to solid media. *gpr3* mRNA levels also responded to the presence of fungal hyphae or cellulose membranes. Silencing of *gpr1* resulted in avirulent mutants unable to attack and over-grow host fungi like *Rhizoctonia solani*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum*. Further analysis of *gpr1*-silenced mutants revealed decreased infection structure formation upon direct contact with *R. solani* which could be restored by the addition of extracellular cAMP. In addition, *gpr1*-sil mutants showed reduced production of the antimicrobial metabolite 6-pentyl-alpha-pyrone, but enhanced formation of chitinases during simulated mycoparasitism.

Analysis of determinants of *Pseudomonas fluorescens* WCS374r involved in induced systemic resistance in *Arabidopsis thaliana*

Mohammad Djavaheri, Jesús Mercado-Blanco, L.C. Van Loon, Peter A.H.M. Bakker..... 109-112

Abstract: The role of iron-regulated determinants of *Pseudomonas fluorescens* WCS374r in mediating induced systemic resistance (ISR) against *Pseudomonas syringae* pv. *tomato* DC3000 (Pst) and *Turnip crinkle virus* (TCV) was studied in *Arabidopsis thaliana*. Under conditions of iron limitation, WCS374r produces large amounts of salicylic acid (SA), the fluorescent siderophore pseudobactin (Psb), and pseudomonine (Psm), a siderophore with an SA moiety. The biosynthesis of SA and Psm in WCS374r is closely related, since mutants impaired in SA biosynthesis are also defective in the production of Psm. Mutants affected in the production of one or more of these metabolites were used to unravel their role in ISR by WCS374r against Pst and TCV. WCS374r-mediated ISR against Pst does not rely on the production of any of the iron-

regulated metabolites, because all mutants impaired in the biosynthesis of one or several of these metabolites elicited ISR. In contrast, SA biosynthesis by WCS374r is a prerequisite to effectively trigger ISR against TCV, and simultaneous biosynthesis of Psb appears to be required. Our data suggest that different determinants of WCS374r are required for ISR against different pathogens.

Mechanisms involved in biocontrol activity of *B. Subtilis* B49b against *Fusarium oxysporum* f.sp. *radicis-lycopersici*

Florica Constantinescu, Gerben Voshol, Guido Bloemberg 113-116

Abstract: Biocontrol of phytopathogenic fungi by *Bacillus subtilis* is accomplished by antibiosis, induced systemic resistance, competition for nutrients and niches or plant growth promotion. This study focuses on mechanisms involved in the biocontrol activity of *B. subtilis* strain B49b against *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl), which causes tomato foot and root rot. *In vitro* and *in vivo* assays demonstrated antagonistic activity of strain B49b against Forl and biocontrol of tomato foot and root rot. B49b was characterized for abilities that might play a role in its biocontrol activity like protease and lactonase secretion, motility and production of antifungal metabolites. TLC and HPLC analyses indicated that strain B49b is producing the antibiotics Iturin A and surfactin. In order to visualize the interaction between *B. subtilis* and Forl hyphae, as well as the ability to colonize the tomato roots, *B. subtilis* B49b was transformed with the *gfp* expressing plasmid pAD44-12 by electroporation. To our knowledge, this is the first report on the successful introduction of a GFP marker gene in an undomesticated *B. subtilis* strain, enabling the direct observation of this promising biocontrol strain on tomato roots *in situ*.

Lipopeptides are key factors in the biocontrol activity of *Bacillus subtilis* towards powdery mildew and bacterial diseases of cucurbits (ES)

Houda Zeriouh, Diego Romero, Laura García-Gutiérrez, Antonio de Vicente, Alejandro Pérez García 117-120

Abstract: Two *Bacillus subtilis* strains, UMAF6614 and UMAF6639, were selected on the basis of their antagonistic activity against a wide variety of bacterial and fungal pathogens and suppression different cucurbit diseases. In biocontrol assays using cell-free supernatants, disease inhibition indexes were similar to those observed for washed cells, and thereby, antibiosis was suggested as the main mode of action. This hypothesis was supported further by chemical analysis of cell free supernatants that revealed the occurrence of surfactin, iturin and fengycin lipopeptides. Transmission electron microscopy (TEM) analyses revealed the ultrastructural effects of these lipopeptides on fungal and bacterial plasma membranes. Biocontrol assays using lipopeptide-deficient *B. subtilis* transformants allowed us to determine a differential role for iturin and fengycin lipopeptides on antifungal and antibacterial activities.

Bacillus lipopeptides as MAMPs for non-pathogenic bacteria perception and defense responses elicitation in plant cells

Guillaume Henry, Marc Ongena, Emmanuel Jourdan, Philippe Thonart 121-124

Abstract: Cyclic lipopeptides (CLPs) from *Bacillus subtilis* are able to stimulate plant protection through the Induced Systemic Resistance (ISR) phenomenon. On tobacco suspension cells, the more active CLP family of surfactins triggered strong, rapid and transient extracellular alkalization and hydrogen peroxide accumulation without inducing either significant cell death or recalcitrant state. These early responses appeared to be dependent on calcium influx and phosphorylation events through protein kinases activation. Moreover, PLA2 was partially implicated in extracellular pH shift. These results highlight for the first time the pattern of early events following CLPs perception by plant cells which could lead to the emission of the systemic signal leading to the induced state of resistance.

Functional analysis of the grapevine defence reaction against *Armillaria mellea* infection and the identification of a Phase Change Antifungal Protein

Michele Perazzolli, Federica Bampi, Andrea Nesler, Silvia Faccin, Anna Maria Ciccotti, Ilaria Pertot, Cesare Gessler, Claudio Moser 125-128

Abstract: Grapevine root rot, caused by the fungus *Armillaria mellea*, is a serious disease of grapevine in some viticulture areas. The commonly used grapevine rootstocks are not resistant and the existing fungicides are ineffective against the disease. Field observations indicated that young plants did not show symptoms of *A. mellea* infection for 3-4 years, suggesting the activation of defence mechanisms. Specific defence genes such as protease inhibitors, a thaumatin and a tumour related protein appeared strongly induced in the rootstock Kober 5BB, 24 h after A.

mellea inoculation, and their induction was validated in repeated experiments *in vitro* and under greenhouse controlled conditions. In order to elucidate the role of these genes in the plant defence response, the full-length coding sequences have been obtained and cloned in a vector suitable for heterologous expression in bacteria. Functional characterization of the purified recombinant proteins demonstrated that the protein homologous to the *Quercus* Phase Change Related protein inhibits *A. mellea* mycelia growth *in vitro*.

Role of phenylalanine ammonia-lyase activity in the induced resistance by plant growth promoting rhizobacteria against bean common blight disease (*Xanthomonas axonopodis* pv. *phaseoli*)

Adem Bozkurt, Hatice Özaktan 129-132

Abstract: Common blight (*Xanthomonas axonopodis* pv. *phaseoli*) (*Xap*) is one of the most important diseases of bean growing areas in Turkey. In this study, 91 strains of nonpathogenic plant growth promoting rhizobacteria (PGPR) from the rhizosphere of healthy bean plants were examined for their ability to reduce bean common blight diseases severity. Three different processes were tested for biocontrol effects of the PGPR strains against *Xap*: a) pod inoculation tests, b) *in vitro* tests and c) *in vivo* pot tests. Out of the 91 strains, 33 were found effective against *Xap* by giving 40 to 70% reduction in disease severity in pod inoculation tests when compared with the pathogen-alone treatment. Antagonistic activity of these PGPR strains was based on the production of fluorescent siderophores in *in vitro* tests. Twelve effective strains of 33, including fluorescent pseudomonads and Gram (+) bacteria were selected according to their results of *in vivo* pod tests. PGPR strains were applied to seeds of the bean in *in vivo* pot trials. All of the selected PGPR strains reduced the diseases severity by the range of 37 to 64% compared with the pathogen alone treatment. Six strains of the selected rhizobacteria were found more promising to inhibit the pathogen than Acibenzolar S-methyl treatment. These results indicated that seed bacterization by PGPR strains could induce systemic resistance in bean plants to *Xap* infection. Phenylalanine ammonia-lyase (PAL) activity was estimated spectrophotometrically in foliar extracts of bean plants grown from seeds that were seed-bacterized with PGPR strains, and inoculated with *Xap*. The level of PAL in foliar extracts increased significantly upon the PGPR treatment according to the pathogen treatment alone. The reduction in the bean common blight disease incidence was found correlative to the amount of increased level of PAL.

Detection of siderophores and indole-3-acetic acid (IAA) production from endophytic fungi, isolated from Thai terrestrial orchid, *Pecteilis susannae* (L.) Rafin

Ruangwut Chutima, Suyanee Vessabutr, Bernard Dell, Pipob Lumyong,

Saisamorn Lumyong 133-136

Abstract: Biocontrol achieved by endophytic fungi and effects of these fungi on plant growth are widely studied due to the need for new sources of biological control agents and plant growth promoters. In this study, 42 endophytic fungi isolated from roots of the Thai terrestrial orchid, *Pecteilis susannae* (L.) Rafin (Orchidaceae), were studied. Sixteen isolated fungi were shown to produce siderophores in solid medium by using the chrome azurol S (CAS) agar plate assay. Twenty-two isolated fungi were shown to produce indole-3-acetic acid (IAA) in culture supplemented with 0.2% (wt/vol) of L-tryptophan. The mycelia sterilia CMU Pec 078 showed the highest productivity of IAA, i.e. 94.7 ppm. L-tryptophan at 0.4% increased IAA production to 112 ppm. Corn seeds (*Zea mays* Linn.) were treated with supernatant of CMU Pec 078 showed 34% seed germination while corn seed germination treated with water, 150 ppm of indole-3-butylic acid (IBA) reagent and 150 ppm of IAA reagent showed 24, 32 and 39% of seed germination, respectively. Our results demonstrate that some of the endophytic fungi isolated from this terrestrial orchid species represent interesting properties for potential use as biocontrol agents and plant growth promoters.

Biological activity of *Pseudomonas syringae* toxins against *Botrytis cinerea* on strawberry fruits

Elisabetta Pellegrini, Carmela Siche, Alberto Fior, Vincenzo Foglian, Ilaria Pertot 137-140

Abstract: Purified syringomycin E (SR-E) and syringopeptin 25A (SP25A) are two lipodepsipeptides produced by *Pseudomonas syringae*. Our aim was to evaluate their efficacy in controlling *Botrytis cinerea*. The direct toxic effect on *B. cinerea* conidia of different concentrations of SR-E and SP25A was tested. The efficacy of SR-E and SP25A against *B. cinerea* on strawberries was evaluated putting small drops (20 µl) of conidial suspension with

toxins or water in the middle of a cross-shaped cut on the surface of strawberry fruits. The number of diseased fruits and the proportion of diseased fruit area were recorded to calculate disease incidence and severity. The effects of the toxins on the fruit skin before fungal development were also observed. Both LDPs reduced the disease, but also caused necrosis areas on strawberry tissues, confirming the double role (plant pathogen and antagonist of pathogens) of this organism.

Biochemical characterization of bacterial antagonists and immunohistochemical changes after resistance induction in tomato against bacterial wilt caused by *Ralstonia solanacearum*

Henok Kurabachew, Kerstin Wydra 141-144

Abstract: A total of 150 rhizosphere bacterial isolates were isolated from tomato and potato rhizosphere soil from Ethiopia. The isolates were screened *in vitro* for their antagonistic effect against the pathogen *R. solanacearum* using dual culture assay. Based on their inhibitory activity, five isolates were selected for *ad planta* experiment with tomato genotypes King Kong2 and L390, moderately resistant and susceptible, respectively. The isolates were identified as *Pseudomonas putida*, *Bacillus cereus*, *Pseudomonas* sp. and *Bacillus* sp. and further characterized for plant growth promoting (PGP) activity such as siderophore, indole acetic acid and hydrogen cyanide production and phosphate solubilization capacity. Selected promising strains suppressed wilt incidence both in split root and pot experiments and improved growth of the plant, indicating the potential for resistance induction and for growth promotion. The alteration of the cell wall structure after infection and after inoculation of the plant with antagonistic bacteria was investigated by immuno-fluorescent microscopy.

Determination of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by GC/MS

Bernhard Kluger, Sylvia Lehner, Norbert Stoppacher, Susanne Zeilinger, Rudolf Kraska, Rainer Schuhmacher 145-148

Abstract: Filamentous soil fungi such as *Trichoderma* spp. are used as biocontrol agents against plant pathogenic fungi (e.g. *Botrytis cinerea*, *Rhizoctonia solani*) in agriculture. *Trichoderma* are able to mycoparasitise phytopathogens. Several different secondary metabolites such as microbial volatile organic compounds (MVOCs) are involved in the mycoparasitic process between *Trichoderma* and the pathogen. In this study we determined MVOCs in cultures of *Trichoderma atroviride* ATCC 74058 using solid phase microextraction (SPME) coupled to gas chromatography/mass spectrometry (GC/MS). In total 50 MVOCs were detected under the tested conditions, thereof 13 were identified by comparison of mass spectra with those contained in commercial libraries and linear temperature programmed retention indices (LTPRI). The detected compounds belong to the classes of alkanes, ketones, mono- and sesquiterpenes and alcohols.

The nutrient status of the pathogen can have an impact on the importance of different biocontrol mechanisms in *Pseudomonas fluorescens* CHA0

Matthias P. Lutz, Geneviève Défago, Monica Maurhofer 149-154

Abstract: Different biocontrol mechanisms are known to be essential for the biocontrol activity of pseudomonads. In *Pseudomonas fluorescens* CHA0 the production of antimicrobial compounds such as 2,4-diacetylphloroglucinol (Phl) is one of the most important biocontrol mechanisms. Strain CHA0 is able to protect cress against *Pythium ultimum* in a gnotobiotic system, where the pathogen is applied as mycelium covered millet. In this system, a *gacA* mutant deficient in the production of various secondary metabolites such as Phl, pyoluteorin and HCN is not able to protect the plants. Therefore, the production of antimicrobial compounds is crucial for the biocontrol activity. We developed a new gnotobiotic system in which *P. ultimum* was amended after cultivation on clay mineral vermiculite with little added nutrients. In this system, a *gacA* mutant was able to protect cress against *P. ultimum* to a similar extend as the wild-type strain CHA0 suggesting mechanisms other than the production of *gacA* controlled metabolites to be important for disease suppression. The reason for this phenomenon might be that if *P. ultimum* is grown under nutrient limiting conditions biocontrol mechanisms such as competition for nutrients or the production of other, not *gacA* controlled metabolites might be sufficient or more important to achieve disease control and *gacA* controlled antimicrobials such as Phl and HCN are not needed. We therefore conclude that the importance of different biocontrol mechanisms in the suppression of *P. ultimum* is dependent on the nutrient status of the pathogen.

Expression profiling of <i>Ampelomyces quisqualis</i> during mycoparasitism of <i>Podosphaera xanthii</i> <i>Alessandro Ferrari, Alberto Ferrarini, Antonio Chimento, Massimo Delledonne, Monica Maurhofer, Ilaria Pertot</i>	155-158
Abstract: <i>Ampelomyces quisqualis</i> is one of the most successful commercial biocontrol agents. It is effective against powdery mildews of several crops, but its activity is often inconsistent. Information on the molecular interactions between the mycoparasite and the host and the effect of the environment on the mechanism of parasitization could help to improve its biocontrol efficacy. The aim of this work was to study gene expression during three different characteristic stages of parasitization: host recognition, active and late parasitization.	
Combining 2,-4-diacetylphloroglucinol and pyrrolnitrin activities in <i>Pseudomonas fluorescens</i> Q8r1-96 increases its ability to suppress <i>Rhizoctonia solani</i> <i>Irit Moseri, Mohammed Bimerew, Marianna Ovadis, Linda Thomashow, Leonid Chernin</i>	159-162
Abstract: <i>Pseudomonas</i> species that produce broad-spectrum antibiotics, including 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (Prn), are currently a focus of biocontrol research. The 5.8-kb <i>prnABCD</i> operon encoding Prn from <i>Pseudomonas fluorescens</i> strain Pf-5 was cloned under control of the <i>tac</i> promoter into the broad-host-range plasmid vector pUCP26 and the integrative vector pBK-miniTn7- Ω Gm. The constructs were used to enhance antifungal activity and broaden the range of target pathogens of the closely related DAPG-producing strain <i>P. fluorescens</i> Q8r1-96. Derivatives of strain Q8r1-96 carrying the <i>tac-prnABCD</i> cassette either on a plasmid or in their chromosome produced Prn in addition to DAPG, as shown by TLC and HPLC analyses, and protected beans against <i>Rhizoctonia</i> root rot under greenhouse conditions significantly better than the parental strain.	
Role of gluconic acid production in phosphate solubilisation, biocontrol ability and rhizosphere competence of <i>Pseudomonas fluorescens</i> CHA0 (Abstract) <i>Patrice de Werra, Maria Péchy-Tarr, L. Rochat, Christoph Keel, Monica Maurhofer</i>	163
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General biological control

Critical factors in the large-scale application of a microbial biocontrol agent in horticultural growing media (NL) <i>Marlies Dissevelt and Willem Ravensberg</i>	179-183
Abstract: Following the selection of a microbial biocontrol agent and its development into a product, effective and reliable field performance under commercial conditions is essential. In this paper critical factors in the large-scale application of a microbial biocontrol agent in horticultural growing media are illustrated with the use of <i>Trichoderma harzianum</i> strain T-22. In the Netherlands a number of challenges appeared with the application through drip irrigation systems in rockwool grown vegetable crops. Irrigation regimes, use of disinfectants (for cleaning the irrigation system) and lack of knowledge of the drip irrigation system negatively influenced the delivery and establishment of the fungus in the rhizosphere. The use of a granular formulation and adjustments in advice on the application method and timing, and the use of disinfectants resulted in an improved establishment of the fungus in the rhizosphere of rockwool grown vegetables.	
<i>Trichoderma atroviride</i> SC1 for biocontrol of fungal diseases in plants <i>Ilaria Pertot, Claudia Maria Oliveira Longa, Federica Savazzini, Lorenza Michelon, Daniele Prodorutti, Chiara Pellegrini, Alessandro Ferrari</i>	185-188
Abstract: <i>Trichoderma atroviride</i> strain SC1 can suppress and prevent the development of plant pathogens, in particular fruits and root rots, such as those caused by <i>Botrytis cinerea</i> and <i>Armillaria</i> spp., powdery mildews, wood diseases (Esca disease). <i>T. atroviride</i> SC1 is a mesophilic fungus as most <i>Trichoderma</i> spp. It is able to utilize a wide range of compounds as sole carbon and nitrogen sources. <i>T. atroviride</i> SC1 survives in a temperature range comprised from -1 and 35°C and grows in a range of temperatures comprised from 5 to 30°C. The optimal temperature for growth is 25 ± 1°C. The maximum temperature for <i>T. atroviride</i> SC1 survival (35°C) is lower than human body temperature, which is a good indication that this fungus is not pathogenic to humans. <i>T. atroviride</i> SC1 survives for more than one year after soil treatments reaching a concentration that is similar to other resident <i>Trichoderma</i> spp. strains. It has only a transient effect on natural soil microflora. These characteristics make it a good candidate for biological control of <i>Armillaria</i> spp. root rots. Promising results were obtained against foliar and	

wood diseases. Higher efficacy could be reached by improving *T. atroviride* SC1 survival in the phyllosphere by using a suitable formulation.

Biocontrol management in soilless culture: impact of the antagonist *Pythium oligandrum* on native fungal populations

Jessica Vallance, Gaétan Le Floch, Franck Déniel, Patrice Rey 189-192

Abstract: Fungal populations and their dynamics were investigated in relation to the introduction of the biocontrol agent *Pythium oligandrum* in the rhizosphere of tomato plants grown in greenhouses. The research was done by inoculating roots of tomato plants grown in hydroponic culture by three selected strains of *P. oligandrum*. After introduction, these strains were monitored over time to evaluate their persistence and their effect on the microflora. *P. oligandrum* was detected at high rates throughout the 8-months experiment by Real-Time PCR but seemed to have a slight influence on the root distribution of the other *Pythium* species, especially on *Pythium* group F, ubiquitous tomato root minor pathogens. Inter Simple Sequence Repeat analysis performed on *P. oligandrum* isolates collected at the end of the growing season, showed that 90% of the samples belonged to only one of the three selected strains. Single-Strand Conformational Polymorphism analyses pointed out that the introduction of the antagonistic fungus only had a slight influence on the native fungal community and its dynamics.

Effects of in vitro antagonists of *Rhizoctonia solani* AG 1-IB on lettuce bottom rot disease and on microbial rhizosphere communities

Rita Grosch, Modupe Adesina, Kornelia Smalla 193-196

Abstract: Bacterial antagonists from suppressive soils were evaluated in growth chamber experiments for their efficacy in controlling bottom rot caused by *Rhizoctonia solani* AG1-IB on lettuce. The ability of the antagonist to colonize the lettuce roots was evaluated first. Whereas six *in vitro* antagonists colonized the rhizosphere of lettuce at comparable rates, only four isolates significantly decreased disease severity in first experiments. Three of them were identified as *Pseudomonas fluorescens* and one as *P. jessenii*. In subsequent experiments involving the four best antagonists, only *P. jessenii* RU47 showed effective and consistent suppression of *R. solani* AG1-IB. The DGGE fingerprints of *Pseudomonas*-specific *gacA* gene fragments from total community DNA showed that the antagonist RU47 established as a dominant *Pseudomonas*-population in the rhizosphere. The total *Pseudomonas* community was not influenced. Furthermore, PCR-DGGE fingerprints of fungal communities indicated a considerable decrease in the relative abundance of the pathogen in the rhizosphere of lettuce when inoculated with RU47. Our results suggest that *P. jessenii* RU47 is a promising biological control agent to suppress *R. solani* AG1-IB in lettuce.

The non pathogenic *Fusarium* isolate Fo47 protects pepper plants against *Verticillium dahliae*

Javier Veloso, José Díaz 197-200

Abstract: *Verticillium dahliae* Kleb. is a pathogen of pepper (*Capsicum annuum*) that causes a great impact on its cultivation. Infection takes place in roots and goes on through the stem, invading the vascular tissues and causing typical symptoms such as dwarfism and wilt. *Fusarium oxysporum* Fo47, a non-pathogenic isolate, was tested as a biocontrol agent against *Verticillium* wilt on pepper plants. Plants were first inoculated with Fo47 and 48 h later with *V. dahliae*. A significant reduction of *Verticillium* wilt symptoms was observed in plants treated with Fo47. Samples of root and stem were collected 48 h after inoculation with Fo47 and 24 h after inoculation with *V. dahliae* to be further analysed. Peroxidase and chitinase enzyme activities were assayed, finding no differences. Likewise, root and stem samples were taken 48 h after inoculation with Fo47 and gene expression was studied by real-time RT-PCR. Two defense-related genes were assayed, encoding a chitinase (CACHI2) and a PR-1 protein (CABPR1). Fo47-treated plants exhibited an increased expression of these genes.

A new biocontrol agent against *B. cinerea* and other plant pathogens

Rudi Aerts, Liesbeth Vogels, Kathleen Heyens, Miguel F.C. De Bolle, Bruno P.A.

Cammue 201-205

Abstract: BCA1, a biocontrol agent isolated from a *Botrytis cinerea* infection on a tomato stem, has been studied for its biocontrol activity. Several plant pathogenic fungi are inhibited in the presence of BCA1. Detailed studies on tomato leaves and stem wounds show that a preventive treatment with BCA1 (non formulated spores) significantly decreases the number of *B. cinerea*

infections. Curative treatment of *B. cinerea* infections on tomato leaves and stem wounds, respectively, reduce the expansion of the infection or even completely stop the infection in optimal conditions. The broad spectrum of BCA1 combined with the complete safety for plants makes a lot of other applications possible including seed disinfection..

Biocontrol of *Armillaria* root rot on highbush blueberry in Italy

Daniele Prodorutti, Alberto Pellegrini, Ilaria Pertot 207-210

Abstract: Plants showing poor growth and blight were found in highbush blueberry orchards in the Trentino region (Northern Italy). Symptomatic plants had rotted roots. Molecular based methods identified two *Armillaria* species (*A. gallica* and *A. mellea*) on symptomatic blueberry roots and on mulching bark. In order to verify the role of bark and wood debris in spreading the infection, *Armillaria* infected bark and wood pieces were added to young blueberry plants. The efficacy against *A. gallica* and *A. mellea* of some potential biocontrol agents was evaluated in laboratory (dual culture) and under greenhouse controlled conditions on strawberry and blueberry plants. Bark and wood pieces inoculated with the two *Armillaria* species infected plants and reproduced *Armillaria* root rot symptoms. The species belonging to *Trichoderma* genus, especially *T. atroviride* SC1, were the most effective biocontrol agents, both against *A. gallica* and *A. mellea*.

Fungal isolates of the order *Sebacinales* provide growth promotion and systemic disease resistance to barley

Monica Sharma, Karl-Heinz Kogel 211-215

Abstract: *Piriformospora indica* is a model organism of the order *Sebacinales* colonizing the roots of various plant species, among them cereals and dicotyledonous plants, including Brassicaceae. The fungus protects barley plants against many root-, leaf-, and ear-infecting pathogens. We studied isolates of the *Sebacina vermifera* complex which have been isolated at different locations worldwide. The phylogenetic relationship between these fungal isolates was determined on the basis of nuclear genes coding for the internal transcribed spacer (ITS1-5.8S-ITS2) region. The isolates were also characterized with respect to their morphological features, such as spore size and hyphal structure as well as growth rate in different media, and further evaluated for their biological potential in barley. All the tested isolates colonized barley roots, induced growth promotion, and elicited systemic resistance against powdery mildew, though with great variations. Our results demonstrate that *P. indica* is the archetype of a novel fungal order that does comprise many fungi with beneficial activities on plants.

Antifungal activity of *Salvia officinalis* extract against *Plasmopara viticola*

Silvia Dagostin, Tiziano Formolo, Oscar Giovannini, Annegret Schmitt, Ilaria Pertot 217-220

Abstract: Plant extracts may represent an alternative to chemical fungicides. The efficacy against grapevine downy mildew of sage (*Salvia officinalis*) alcoholic extract, its persistence and rain fastness were evaluated. When applied on artificially inoculated potted grapevine plants under greenhouse controlled conditions the sage extract provided high control of disease, whereas in field experiments the efficacy was lower. The residual activity (persistence) of the sage extract is good, but it is easily washed away by rain. In facts even 10 mm of simulated rain significantly reduce its control activity. With a suitable formulation, able to reduce the rain fastness, this sage extract could be a promising control tool for organic viticulture.

Control of *Ralstonia solanacearum* in *Curcuma alismatifolia* Gagnep. by antagonistic bacteria

Saran Promsai, Narumol Thongwai 221-224

Abstract: Six isolates of *R. solanacearum*, namely PRZ, PT1B, PT1J, RRD, RT1S and D1, were isolated from infected patumma rhizomes. Their high infectivity was confirmed by re-infection into healthy patumma rhizomes which showed wilt symptoms upon direct infection. All six bacterial pathogen isolates were used as test microorganisms for screening of antagonistic bacteria by using an agar disc diffusion test. It was found that among seventy-one bacterial isolates isolated from soil collected from Thailand, five bacterial isolates SR15, SR38, SR46 and SR58 demonstrated high efficiency growth inhibition against all six *R. solanacearum* isolates.

In vitro and in planta suppression of oncogenic strains of *Agrobacterium vitis* and *Agrobacterium tumefaciens* by bacterial biocontrol agents

Natalia Dandurishvili, Ernő Szegedi, Pikria Eliashvili, Naili Giorgobiani, Inessa Khmel, Alexander Vainstein, Leonid Chernin 225-229

Abstract: *Agrobacterium vitis* and, to a lesser extent, *A. tumefaciens* are the cause of crown gall on grapevines, which is a major concern to grape growers worldwide. Several bacterial antagonists demonstrating the ability to suppress *In vitro* growth of oncogenic strains of *A. vitis* and *A. tumefaciens* isolated from vineyards in Hungary and Georgia were tested for their ability to prevent gall formation on tomato seedlings infected by these pathogens. The antagonists were applied together with the pathogens, 2 or 7 days before infection. The 2,4-diacetylphloroglucinol (DAPG)-producing *Pseudomonas fluorescens* strain Q8r1-96, known as an efficient biocontrol agent of *Gaeumannomyces graminis* var. *tritici* which causes take-all disease of wheat, was found to protect tomato plants against crown galls as well. The effect was manifested by a strong reduction in gall fresh and dry weights and size. Significant control of galls was also achieved with *P. fluorescens* strain B-4117 producing DAPG, pyrrolnitrin (Prn) and pyoluteorin and with *Serratia plymuthica* strain IC1270 producing Prn. An avirulent strain of *A. tumefaciens* also protected tomato against oncogenic strains of *A. tumefaciens* and *A. vitis*. Volatile organic compounds produced by *Serratia* and *Pseudomonas* strains suppressed growth of pathogenic agrobacteria *in vitro*.

Biological control of avocado root rots by suppressive organic amendments

Nuria Bonilla, Juan Antonio Torés, José María Hermoso, Jorge González, Francisco Manuel Cazorla, Antonio de Vicente 231-234

Abstract: In Southern Spain, the most destructive avocado root rot diseases are mainly caused by the fungus *Rosellinia necatrix* and the oomycete *Phytophthora cinnamomi*. Different approaches have been used for controlling avocado root rot diseases. Among them, the addition of organic amendments or mulches is one of the most popular actions performed by farmers. In order to test the suppressive ability of different organic mulches against those soil-borne pathogens, different greenhouse experiments of biocontrol were designed. The suppressive effect against *R. necatrix* was tested using two-year-old avocado plants growing in pots with soil supplemented with different organic amendments. Results demonstrated a variable degree of reduction of the disease index in the plants treated with organic amendments in comparison with non-amended plants. To test the potential suppressive effect against *P. cinnamomi*, greenhouse experiments were performed using six-month-old avocado plants germinated and grown in soil treated with the different mulches.

Trichoderma in Brazil: history, research, commercialization and perspectives

Wagner Bettiol, Marcelo A.B. Morandi 235-237

Abstract: In 1987, thirty-seven years since the first publication on biocontrol of plant diseases by *Trichoderma* in Brazil, a pioneer product arrived on the market against *Phytophthora cactorum* in apple trees. At that time, the biocontrol agent (BCA) used to be supplied in polypropylene bags containing 24 g of sorghum seeds colonized by *Trichoderma viride*. The first enterprise specialized in production and commercialization of *Trichoderma* started to operate in 1992. Since then, other products came out and nowadays there are more than ten commercial trademarks. In April 2008, we did a survey to check the state-of-the-art of the use of *Trichoderma* in Brazil and verified: (1) main species in the market are *T. asperellum*, *T. harzianum*, *T. stromaticum*, and *T. viride*; (2) pathogen targets include *Fusarium*, *Pythium*, *Rhizoctonia*, *Macrophomina*, *Sclerotinia*, *Sclerotium*, *Botrytis*, and *Crinipellis perniciosus*; (3) recommended crops are bean, soybean, cotton, tobacco, strawberry, tomato, onion, garlic, ornamentals, and cacao; (4) *Trichoderma* are mostly produced by solid fermentation on rice or millet grains (approximately 550 ton/year); and (5) formulations include wettable powder and granules, suspension concentrates, emulsion oil, grain+spores, and dry spores. The average cost of treatment, for example, against bean white-mold with *Trichoderma* is US\$ 54.00/ha while with fungicides is about US\$ 92.00/ha. The area treated with *Trichoderma* has highly increased during the last three years. The recent organization of a Brazilian Biocontrol Association and the enhancement of the legislation for registration and commercialization of BCAs are boosting the market, particularly for *Trichoderma* that is in frank expansion.

Control of *Fusarium* in chrysanthemum with sewage sludge, biofertilizer, hydrolyzed fish, chitosan, and *Trichoderma*

Wagner Bettiol, Zayame Vegette Pinto 239-242

Abstract: *Fusarium oxysporum* f.sp. *chrysanthemi* can cause severe losses in chrysanthemum (*Chrysanthemum morifolium*) in Brazil. This study was done to evaluate the efficacy of sewage sludge, biofertilizer, hydrolyzed fish, chitosan, and *Trichoderma harzianum* to control *Fusarium* in chrysanthemum in substrate composed of pine bark (pH 5.5; EC 0.6 μ S) obtained from pots of dead chrysanthemum plants. The infested substrate was or was not sterilized in water vapor (2 h; 100°C); with sewage sludge incorporated (0, 10, 20, and 30% v/v). Into these mixtures were added (or not) biofertilizer (14 ml/l), hydrolyzed fish (10 ml/l) and *Trichoderma* (10^8 conidia/ml), transferred to pots (3 l) and planted with 'Yellow-Marino' chrysanthemum seedlings. For all treatments, half of the pots were sprayed with chitosan (200 mg/l) weekly. A multifactorial randomized experimental design with 24 replications, totaling 2560 pots, was adopted. The plants were grown in a commercial greenhouse and evaluations for disease severity (1=healthy plant; to 5=dead plant or wilted leaves) were performed at 8, 12, 14, 18 weeks after transplanting. In addition, plants were classified in commercial (class I, II, III) or not, after 18th week. The level of disease control was directly proportional to sewage sludge concentration incorporated in the substrate. Biofertilizer, hydrolyzed fish, chitosan and *Trichoderma* did not control the disease. In general, disease severities were higher in plants growing in sterile substrate when compared the plants growing in non-sterile substrate.

Biological control of *Sclerotinia sclerotiorum* on beans in field by *Trichoderma asperellum* and *Clonostachys rosea*

Marcelo A.B. Morandi, Lucio B. Costa 243-246

Abstract: White mold (*Sclerotinia sclerotiorum*) is a destructive disease of bean crops in winter in Brazil. Biocontrol agents (BCAs) are being tested against the pathogen, including species of *Trichoderma* and *Clonostachys rosea*. The objective of this work was to evaluate the effectiveness of one isolate of *T. asperellum* and one of *C. rosea* previously selected in controlled conditions against the white mold in an irrigated field during a winter crop. The experiment was composed of 36 micro-plots (1 m² each) severely infested with sclerotia in a previous bean crop. There were six treatments: check (no sclerotia), infested check, fungicide (Fluazinan), *T. asperellum*, *C. rosea* I62, and Trichodermil (commercial product). We observed a significant reduction in apothecium emergence in all plots treated with BCAs and with fungicide. The incidence and severity of the disease were only marginally reduced in the biocontrol treatments. Although the intensity of the disease was significantly reduced in the fungicide plots, no differences were observed in the yield among treatments. These results are probably due to the high level of ascospores produced in the check and disseminated to the other plots. The observed reduction in apothecium counts on treated plots indicates the potential of the BCAs to reduce the survival and multiplication of the pathogen in field along time.

Natural biological control of chestnut blight in Croatia

Ljiljana Krstin, Sanja Novak Agbaba, Daniel Rigling, Mirna Ćurković Perica 247-250

Abstract: The chestnut blight fungus *Cryphonectria parasitica* has been responsible for the decline of chestnut trees in Croatia for five decades. In order to investigate whether virus-induced hypovirulence is naturally present in all *C. parasitica* populations or human-mediated biological control is necessary, we sampled 338 fungal isolates from 10 chestnut populations throughout chestnut-growing coastal and continental areas of Croatia. Altogether, 18 vegetative compatibility (vc) types were identified. Perithecia and both mating types of *C. parasitica* in approximately 1:1 ratio were found in all populations suggesting sexual reproduction of the fungus. Although quite high vc type diversity and sexual reproduction were confirmed, which usually presents an obstacle for the spreading of the hypovirus, natural hypovirulence was evident in all populations. Incidence of hypovirus-infected isolates ranged from 13% in coastal to 67% in the continental part of the country. All viral isolates belonged to the Italian subtype of *Cryphonectria* hypovirus 1 (CHV-1) and were closely related to the isolates found in other European countries.

Antagonistic potential of *Trichoderma* isolates against soil and seed borne pathogens in vitro

Asha Shivpuri, S.N. Mali..... 251-254

Abstract: Twenty one *Trichoderma* isolates of five *Trichoderma* spp. were obtained from the rhizosphere soil of cotton, groundnut, mustard and soybean crops. Of these, six were *T. harzianum*, eleven were *T. viride*, two of *T. aurioviride*, one *T. polysporum* and one was of *T. koningii*. Two isolates of *T. virens* were collected from the Integrated Pest Management Lab of Government of Rajasthan, Durgapura, Jaipur. All the 23 isolates were tested against soil and seed borne pathogens viz. *Fusarium oxysporum* (wilt of cotton), *Aspergillus niger* (collar rot of groundnut), *Sclerotinia sclerotiorum* (stem rot of mustard) and *Rhizoctonia bataticola* (root rot of soybean) using dual culture technique. Studies revealed that *T. harzianum* from soybean (S-9) and *T. viride* from groundnut (G-10) and from cotton (C-23) were highly antagonistic to *A. niger* inhibiting 78 to 85% growth of pathogen. For *F. oxysporum*, *T. viride* from mustard (M-8) and *T. harzianum* (S-12) from soybean were found best in inhibiting the growth of pathogen (71-88%). Results revealed that *T. viride* from cotton (C-6) was highly antagonistic to *Sclerotinia sclerotiorum* causing stem rot in rapeseed mustard. Similarly against *R. bataticola* isolates of *T. virens* (Tv₁ and Tv₂) obtained from IPM Lab were found effective with 61.1% inhibition. As the temperature in the state goes above 40°C in the month of June-July (sowing season of groundnut, soybean and cotton). Therefore it is an important issue to see the viability of these isolates at different temperatures. Experiments results revealed that isolates of *T. viride* (C-5, C-23, G-10 and S-11) could grow profusely and conidiate well at 40°C after 72 and 96 hrs of incubation. These isolates will be tested against seed/soil borne pathogens of groundnut, cotton, soybean and mustard.

Inhibition of cotton pathogens by natural antagonists

Rustam Mannanov, Rano Sattarova 255-258

Abstract: Three antagonistic bacteria, *Pseudomonas fluorescens* 41, *Bacillus subtilis* 23 and *Bacillus megaterium* 26, were tested in vitro for their ability to control *Xanthomonas malvacearum*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *vasinfectum* and *Verticillium dahliae*, which are agents of major cotton diseases. *Bacillus subtilis* 23 was the most active against phytopathogens in laboratory experiments and in small-plot trials. In field trials, the biological efficacy of pre-sowing cotton seed treatment with the most effective antagonist was studied on Upland variety of cotton *Gossypium hirsutum* C-6524. Pre-sowing seed treatment with *Bacillus subtilis* 23 showed significant inhibition of the development of *Xanthomonas malvacearum* and *Rhizoctonia solani* as well as stimulating effect on cotton yield. The highest control efficacy (64%) was recorded in case of *Bacillus subtilis* 23 against *Rhizoctonia solani*.

Use of *Trichoderma atroviride* SC1 inoculated barks to control *Armillaria* root rot in highbush blueberry orchards

Alberto Pellegrini, Daniele Prodorutti, Chiara Pellegrini, Thomas Paternoster, Veronica Leoni, Ilaria Pertot 259-262

Abstract: *Armillaria* root rot on highbush blueberry is an increasing problem in Trentino region (northern Italy) causing decline and consequent death of plants. Coniferous bark is largely used as mulch in blueberry orchards to suppress weeds and maintain low soil pH. Once infected by *Armillaria* spp., this bark could spread the disease in previously disease-free orchards. *Trichoderma atroviride* SC1 is a biocontrol agent of several plant diseases, *Armillaria* root rot included. The pre-treatment of bark with *T. atroviride* SC1 could represent a control tool against *Armillaria* root rot. The aim of this research is to identify growth conditions to improve persistence and viability of *T. atroviride* SC1 on bark used as mulch and to evaluate the efficacy of the *T. atroviride* SC1 inoculated bark to control *Armillaria* spp. The growth of *T. atroviride* SC1 on several substrate/bark combinations was evaluated at different times by counting the colony forming units on a semi-selective medium. Significant differences were found among the various substrate/bark combinations and coniferous bark. In particular larch bark were proved to be a suitable medium to increase *T. atroviride* SC1 persistence.

Double role of *Metschnikowia fructicola*: biocontrol agent of *Botrytis cinerea* and repellent to *Lobesia botrana*

Marco Tasin, Carmela Sicher, Alketa Zeqiri, Vito Simeone, Ilaria Pertot 263-266

Abstract: *Metschnikowia fructicola* is an effective biocontrol agent of postharvest diseases of grapes. It can antagonize *Botrytis cinerea* on several crops. The aim of our work was to investigate the interactions between grape protected by *M. fructicola* and the grapevine moth *Lobesia botrana*. The egg laying preference of *L. botrana* was tested in a dual choice oviposition test. Biocontrol efficacy of *M. fructicola* against *B. cinerea* was evaluated on berries artificially inoculated with the pathogen. *M. fructicola* significantly controlled *B. cinerea* when applied before the artificial inoculation of the pathogen. Females of *L. botrana* preferred to lay their eggs on a healthy grape rather than on a grape inoculated with *M. fructicola*. *B. cinerea* infected berries were slightly attractive, less than healthy ones. Under the conditions of these experiments *M. fructicola* acted both as a biocontrol agent of *B. cinerea* and as a repellent towards *L. botrana*.

Alternative biological treatments against *Rhizoctonia solani* in radish

Francois Lefort, T. Mormentyn, G. Calmin 267-270

Abstract: *Rhizoctonia solani* Kühn is a pathogenic basidiomycete often responsible of damping-off diseases in many plant species and especially in commercial radish culture (*Raphanus sativus* L.). The use of chemical fungicides is the only efficient control method, though no chemicals are authorized in Switzerland against this fungus in radish cultures. The objectives of this work were to evaluate alternative treatments which could be efficient against *R. solani* in greenhouse culture conditions. Experiments were carried out in greenhouse and radish cultures were artificially infected with a strain of *R. solani*. Infected tests were then treated with 7 different preparations: chili pepper powder; *Curcuma longa* powder, *Trichoderma harzianum*, *Aloe vera* decoction, citrus oil, *Datura metel* leaf decoction, oregano decoction. Treatments were compared to the chemical fungicide Rhizolex and to untreated controls. Qualitative and quantitative observations showed a real efficiency of some alternative treatments (chili pepper, *C. longa*, oregano) but only the chemical fungicide was able to stop and control totally severe attacks of the pathogen

Microorganisms associated with *Chara hispida* L. show a high antagonistic potential towards bacteria

Gabriele Berg, Martin Hagemann 271-275

Abstract: Members of the genus *Chara* are multi-cellular green-alga, common lake inhabitants and fulfil important functions in these ecosystems. Little is known about the associated microorganisms of these abundant water inhabitants. To analyse bacteria and fungi associated with *Chara*, samples of *Chara hispida* L. (Bristly Stonewort) were taken from lakes in Mecklenburg-Pomerania (Germany) and analysed by a polyphasic approach including cultivation-dependent and independent techniques. The stoneworts were colonised by bacteria in high ($3 \times 10^4 - 4 \times 10^5$ CFU g⁻¹ fw) and by fungi (10² CFU g⁻¹ fw) in much lower abundances. In general, the isolates showed a low antagonistic potential against fungi (2%); only four out of 180 showed activities against *Verticillium dahliae*. In contrast, a higher proportion of isolates (21%) was active against *Xanthomonas campestris*. In addition, some antagonists obtained displayed a high antagonistic potential against cyanobacteria and no potential against Bacillariophyta. Representatives of each ARDRA group were identified by partial sequencing of the 16S rRNA. *Pseudomonas fluorescens*, *Pseudomonas graminis*, *Enterobacter amnigenus*, *Pantoea agglomerans* and *Bacillus* spp. were identified as prominent antibacterial antagonists. Comparing the microbial community and single antagonistic isolates by SSCP (single strand conformation polymorphism analysis), we found a high diversity of *Chara*-associated bacterial communities and bacterial-antagonists as dominant members of them.

Use of a bacterial antagonist for the biological control of bacterial leaf/fruit spot of stone fruits.

Enrico Biondi, Davide Dallai, Agostino Brunelli, Carlo Bazzi, Emilio Stefani 277-281

Abstract: Bacterial leaf/fruit spot and canker of stone fruits, caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*), is a recurrent disease in Italian orchards. Control strategies are based on several copper treatments, frequently resulting in phytotoxicity and development of copper resistant strains. The need to implement more effective strategies to control the disease led to choose and study bacterial epiphytes for their ability to control *Xap* populations during the growing season, especially from fruitlet development to harvesting. Among a collection of bacterial antagonists,

one was chosen, for its effective inhibition of *Xap in vitro*. The antagonist was identified as a strain belonging to the *Pseudomonas fluorescens* group and able to interact during the infection processes in different host-pathogen systems. During field trials in 2006 and 2007, the antagonist significantly lowered the disease severity. Moreover, experiments done under glasshouse with the antagonist, in parallel with two “bio-stimulants” (2008), resulted again in a remarkable control of the disease.

Field studies on biological control of the walnut blight

Bernard J. Blum, Isabelle Chavignier, William Chauvin 283-286

Abstract: Walnut blight is the most devastating disease complex affecting walnut orchards in almost all production areas, and *Xanthomonas arboricola* p.v. *juglandis* is the most important pathogen involved. Since the conventional use of copper derivatives provides a very poor protection, Agrometrix ICM, in collaboration with several biocontrol products manufacturers and the Station Expérimentale de la Noix in Creysse, France, undertook several field studies in commercial orchards in order to select new biologically-based protection strategies. The studies, conducted over 7 years (2000-2007), demonstrated that additional to the action of *Xanthomonas* on nuts, several pathogens that remain to be conclusively identified induce a Brown Apical Necrosis (BAN) often ignored in the past. The screening of potential biocontrol agents demonstrated that bacterial antagonists may provide an effective protection, but these must be integrated into a protection strategy that is adapted to the causal pathogens and using micro local meteorological records made inside the orchards. Since 2008, studies are being undertaken for the development of an electronic decision supporting tool.

Bacteriophages for biocontrol of *Erwinia amylovora* (Abstract)

Lars Fieseler, Y. Born, Brion Duffy, M.J. Loessner 287

Abstract only

Biological control of cucurbit powdery mildew by plant growth-promoting rhizobacteria (Abstract)

L. García-Gutiérrez, D. Romero, Houda Zerrouh, A. de Vicente, A. Pérez-García 288

Abstract only

Characterization of the potential biocontrol organism *Pantoea agglomerans* 48b/90 (Abstract)

Ulrike Sammer, Beate Völksch, Dieter Spiteller 289

Abstract only

Antagonist-plant-microbial/abiotic environment interactions

Molecular mechanisms involved in controlling nematode infection in crops by introducing mutualistic endophytic fungi

Alexander Schouten, Andreas Kurtz, Andrea Ditzer, Richard A. Sikora 293-296

Abstract: The antagonistic potential of non-pathogenic *Fusarium oxysporum* towards plant pathogenic *F. oxysporum* is well documented. Recently, it was also demonstrated that specific endophytic non-pathogenic *F. oxysporum* strains can successfully reduce nematode infection in banana and rice. Our current molecular and biochemical research aims at understanding the mechanisms involved in this biocontrol system. At the fungal side, we genetically characterize *F. oxysporum* biocontrol isolates. Phylogenetic analysis is performed using sequence information of specific genomic regions to determine whether they can be discriminated from other *F. oxysporum* isolates that do not support biocontrol or can even be pathogenic for the plant. At the plant side, we try to understand the mechanisms leading to biocontrol. Although the biocontrol is systemic and it is assumed that the fungus initiates certain plant defence responses, the exact mode of action is currently poorly understood. We therefore further dissect the defence mechanisms involved using molecular approaches. As a start, we want to determine whether induced systemic resistance (ISR) or systemic acquired resistance (SAR) play a key role in the defence mechanism. In addition, biochemical tools are used to finding alterations in the composition of the root exudates in the presence of the endophyte, which may lead to a reduced attraction or increase in repellency regarding the nematode.

Understanding multitrophic interactions to facilitate successful biocontrol of plant parasitic nematodes with *Paecilomyces lilacinus* strain 251

Sebastian Kiewnick 297-299

Abstract: The facultative egg-pathogenic fungus *Paecilomyces lilacinus* strain 251 (PL251) is one of the most widely tested biocontrol agents for control of plant-parasitic nematodes. Recently, PL251 was included as active substance in Annex I to the directive 91/414/EEC. In the USA, PL251 is registered as bio-nematicide under the trade name MELOCON[®]WG for use on a variety of crops. So far PL251 has demonstrated efficacy in reducing root-knot, cyst and free living plant-parasitic nematodes on a range of crops. However, to better understand the multitrophic interactions of PL251 with host- or non-host plants, nematodes, mutualistic fungal endophytes, and mycorrhiza studies were conducted to determine their importance for biological efficacy. In none of the studies conducted, adverse effects on mutualistic fungal endophytes, mycorrhiza, fungal antagonists or entomopathogenic nematodes were observed. Conversely to other nematophagous fungi, rhizosphere competence seems not a key factor for the efficacy of PL251. However, studies are underway to determine the eggmass colonisation by PL251 using realtime PCR assays which are able to detect 10 CFU per eggmass or less. Monitoring the persistence of PL251 under field conditions using dilution plating techniques and nested PCR revealed a rapid decline of the fungal density in soil over time. Although detection of PL251 in soil was still possible two years after application, the overall suppressiveness of egg pathogenic fungi towards cyst nematodes was not affected.

Defense pathways activated by *Bacillus mojavensis* isolate 203-7 and *B. mycooides* isolate BmJ as elucidated by *Arabidopsis* mutants

Oliver T. Neher and Barry J. Jacobsen 301-305

Abstract: The biological control agents (BCAs), *Bacillus mojavensis* 203-7 and *B. mycooides* BmJ have provided disease control by induced resistance in multiple pathosystems but they differed in effectiveness in different pathosystems. An *Arabidopsis thaliana* mutant–*Botrytis cinerea* pathosystem was used to investigate the plant defense pathways activated by 203-7 and BmJ. A *thaliana* wild type (Col-0) and mutants jar1-1, npr1-5, ein2-1, and NahG, were induced by application of washed cells of the BCAs, sterile dH₂O, the chemical inducers acibenzolar-S-methyl, methyl jasmonate, ethephon or probenazole or specific buffer used to apply chemical inducer. Both BCAs reduced disease severity on wild type and NahG mutants but provided no disease reduction on jar1-1 indicating that induction was salicylic acid independent but jasmonic acid dependent. BmJ did not decrease disease severity on npr1-5, jar1 or ein2-1 mutants. 203-7 induced plants had lower disease severity on npr1-5 and ein1-2 mutants but were equivalent to buffer controls on jar1-1 mutants. Enzyme assays confirmed induction of chitinase, β 1,3 glucanase and superoxide dismutase (SOD) by 203-7 and BmJ. These results demonstrate that induction of induced resistance by BmJ is salicylic acid independent and both NPR1 and ethylene dependent, induction by 203-7, however, jasmonic acid dependent and NPR1 independent.

Beneficial *Pseudomonas* spp. have altered root colonization on *Arabidopsis thaliana* mutants affected in the expression of induced systemic resistance

Rogier F. Doornbos, L.C. Van Loon, Peter A.H.M. Bakker 307-310

Abstract: A select group of non-pathogenic rhizobacteria can elicit induced systemic resistance (ISR) in plants. *Pseudomonas*-mediated ISR is phenotypically similar to pathogen induced systemic acquired resistance (SAR) in that upon challenge inoculation, pathogen proliferation is restricted and disease severity is reduced. We investigated whether the ability to express ISR and/or SAR also affects the non-pathogenic and beneficial rhizosphere microflora. DGGE analysis revealed that mutants of *Arabidopsis thaliana* affected in the ISR and/or SAR signal transduction pathway developed a bacterial rhizosphere microflora that was different from the one on the wild-type control. In order to study possible effects of selected *Arabidopsis* mutants on fluorescent *Pseudomonas* spp., rhizosphere colonization by *P. putida* WCS358r and *P. fluorescens* strains WCS417r and WCS374r was determined. Colonization of WCS374r in the rhizosphere of the *Arabidopsis* Col-0 wild type was lower as compared to the colonizing ability of WCS358r and WCS417r. Root colonization of WCS358r and WCS417r was reduced on *myb72*, a mutant of *Arabidopsis* that can not express ISR. Whereas colonization of WCS358r and WCS417r was not affected on the *sid2* mutant that is impaired in salicylic acid (SA) production, colonization by WCS374r was increased on this mutant. Thus, while the superior colonization

capacity of WCS358r and WCS417r was dependent of *MYB72* expression, the inability of WCS374r to effectively colonize the Arabidopsis rhizosphere appeared to depend on the production of SA by the plant.

Absolute quantification of specific plant defense pathways and disease development of several microbial pathogens on Arabidopsis using real-time PCR

Benoît Boachon, Jérôme Robert, Xavier Daniel, Brigitte Mauch-Mani..... 311-316

Abstract: Monitoring specific plant defence pathways during pathogen infection by measuring the expression of marker genes has been widely used to understand plant microbe interactions. Different methods are used despite the lack of sensitivity and the difficult task one is faced with the normalising of data based on the expression of traditional housekeeping genes which does not remain constant, especially during plant microbe interactions. Moreover, quantification of disease development is usually based on visual observations that are subjective or based on time consuming methods that require many replicates. Here, we present a novel method to measure the implication of specific plant defence pathways during infection coupled to the quantification of *Pseudomonas syringae*, *Hyaloperonospora arabidopsis* and *Botrytis cinerea* growth in plant. Absolute quantification by real-time PCR for each marker or pathogen was done using a standard curve of a well defined cloned gene product. The expression of marker genes and the quantification of pathogen infection were normalised between samples by the sensitive quantification of cDNA and DNA, respectively. This method could be applied to other plant species and pathogens.

Influence of rhizosphere-specific parameters on surfactin production by Bacillus subtilis

Marc Ongena, Venant Nihorimbere, Patrick Fickers, Philippe Thonart..... 317-320

Abstract: Understanding which environmental factors are important and how they influence antibiotic production by beneficial rhizobacteria is a key for improving the level and reliability of biocontrol in disease suppression. Direct evidence for their role in disease suppression can be provided by the demonstration of an efficient production *in situ*. In this work, the production of surfactin that retains several biocontrol functions, by *Bacillus subtilis* growing on tomato roots was studied by using the lacZ reporter system for *psrA* promoter expression and LC-MS quantification. Our results demonstrate that efficient lipopeptide synthesis may occur in cells developing very slowly on roots in poorly aerated soils where available substrates for growth mainly derive from plant exudates.

Genotype-level interactions determine the degree of reduction of leaf rust on wheat by seed application of beneficial pseudomonads

Abbas Sharifi-Tehrani, Stefan Kellenberger, Mohsen Farzaneh, Maria Pechy-Tarr, Christoph Keel, Fabio Mascher 321-325

Abstract: Some bacteria have the capacity to reduce incidence and severity of plant diseases either by inhibiting the pathogen or by modulating the resistance response of the plant. Plants dispose of different resistance mechanisms that are influenced by the biotic and abiotic environment. The present experiments explored the effects of biocontrol strains of *Pseudomonas fluorescens* on the resistance of wheat varieties against brown rust disease caused by *Puccinia triticina*. Root inoculation with biocontrol pseudomonads reduced the disease severity on the leaves. The plant response depended on the genotype of both the microbes and the wheat varieties, suggesting a straight interaction at the molecular level.

cDNA-AFLP analysis of Candida oleophila (strain O) genes differentially expressed during the biocontrol of Botrytis cinerea on harvested apples

Mohammed Bajji, M. Haïssam Jijakli..... 327-330

Abstract: Genes potentially involved in the biocontrol activity of *Candida oleophila* strain O against *Botrytis cinerea* on stored apples were identified by cDNA-AFLP. Gene expression of strain O in the absence (O) and in the presence (OB) of *B. cinerea* spores was compared after 7 h of incubation at 25°C on apple wounds. A total of 1467 (O) and of 1214 (OB) cDNA fragments were visualised using 15 primer pair combinations, the average number of fragments per primer pair being respectively 105 and 87. Of these fragments, 73 were differentially expressed in O compared to OB: 61 down-regulated (4.2%) and 12 up-regulated (1%). Among them, nine sequenced fragments were subjected to real-time RT-PCR to confirm their differential expression. Confirmation was observed for three down-regulated genes (mitochondrial inner membrane

transporter, NADP(+)-dependent dehydrogenase, and alcohol dehydrogenase 2) and one up-regulated gene (acid phosphatase).

Are type III secretion systems found in fluorescent pseudomonads involved in plant colonization or biocontrol?

Fabio Rezzonico, Davide Gobbin 331-335

Abstract: We screened a collection of well-characterized biocontrol *P. fluorescens* strains for the presence of a type III secretion system (T3SS) and analyzed its influence on biocontrol performance in two different pathosystems. Even if the occurrence of the T3SS is *per se* a neutral feature with respect to plant protection, we showed that it is highly correlated with other beneficial traits such as phloroglucinol production. Furthermore, tomato plants showed an improved ability in selecting for T3SS⁺ isolates compared to other plant species tested suggesting that the secretion machinery may be used by biocontrol bacteria for plant-species specific colonization of the rhizosphere.

Molecular tools to analyze the influence of environmental factors on the genetic diversity and the plant-beneficial activity of root-colonizing pseudomonads (Abstract)

Joana Meyer, P. de Werra, M. Frapolli, Maria Péchy-Tarr, L. Rochat, Christoph

Keel, Monica Maurhofer 336

Abstract only

Investigation of primed physiological status of grapevine plantlets (*Vitis vinifera* L.) induced by PGPR *Burkholderia phytofirmans* strain PsJN under low non-freezing temperatures (Abstract)

A. Theocharis, F. Baillieul, Helene Lacroix, J. Nowak, C. Clément, E. Ait Barka 337

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The role of phytohormones in basal resistance and *Trichoderma*-induced systemic resistance to *Botrytis cinerea* in *Arabidopsis thaliana* (Abstract)

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Molecular ecology and impact assessment

The fungal community associated with *Vitis vinifera*: development of molecular probes for observation of its evolution in symptomatic and asymptomatic wood

Valérie Hofstetter, Leonardo Casieri, Katia Gindro, Olivier Viret 343-346

Abstract: Studies on fungi associated with *Vitis vinifera*, essentially focused on esca symptomatic plants, have shown that many and systematically diverse fungi are present in diseased wood. Among these fungi, several have also been isolated from healthy grape plants. The shift from a saprotrophic/endophytic to a pathogenic behaviour suggests that the fungal community associated with grapes is unbalanced through time. To investigate the fungal community associated with *Vitis vinifera*, we have chosen an esca sensitive cultivar (Chasselas, rootstock 3309) to isolate fungi growing from both surface-sterilised symptomatic and young asymptomatic wood. The isolates were characterized based on morphology and ITS sequences. Our molecular classification is based on four loci. Fungal communities retrieved from symptomatic and asymptomatic grapes are dominated by Sordariomycetes, mainly Hypocreales and Xylariales, but they differ among each other partially in their respective species composition. Using both our own sequences and existing data produced by the project 'AFTOL', we also explored the potential of the sequenced genes to design molecular tools for different systematic ranks to study the evolution of the fungal community in wood.

Comparison of effects of chemical and microbial biocontrol agents on non-target microbial soil organisms

Jacqueline W.A. Scheepmaker 347-349

Abstract: The evaluation of non-target effects on soil (micro)organisms is one of the data requirements in registration dossiers of both microbial biological control agents (mBCAs) and chemical control agents (CCAs). No reports are available on comparisons between mBCAs and CCAs concerning their non-target effects. Goal of the present study is to investigate whether the impact of mBCAs on microbial communities differs from the impact of CCAs. Data from studies with agents applied as herbicides, fungicides, insecticides, fumigants, antagonist and plant growth promoting rhizobacteria (PGPR) were included in a data sheet. Data is analysed in a meta-analysis. This desk research is still in process, but first results seem to indicate that non-target effects of mBCAs are shorter of duration compared to effects of CCAs.

Grapevine-associated microorganisms: Antagonistic potential towards *Botrytis cinerea* varies between habitats, cultivation methods and grapevine species

Florian Schmid, Gabriele Berg 351-354

Abstract: *Botrytis cinerea* is a serious fungal pathogen of grapevine all over the world. To develop effective strategies in biological control it is important to understand the microbial ecology of grapevine. Dual culture plate assays were conducted to find out the distributions of the antagonistic potential in different habitats and different plant varieties. In this study, the abundances of antagonists in cultivated grapevine, organically and conventionally treated, were compared to wild varieties of *Vitis vinifera sylvestris* and *V. riparia*. Significant differences of the occurrence and composition of bacterial and fungal antagonists between the different habitats and plant varieties were found.

Detection and differentiation of antagonistic *Erwinia* species by PCR and MALDI TOF analyses

Klaus Geider, Isabel Gehring, Annette Wensing, Anja Freiwald, Sascha Sauer .. 355-358

Abstract: Differentiation and identification of *Erwinia* species was done with specific PCR primers mainly from house keeping genes and EPS encoding regions. Within a species, strains can be distinguished by single nucleotide polymorphisms (SNP) of many genes with non-matching bases and modified conditions for PCR. Classification of isolates was done by nucleotide sequence analysis and alignments of 16S rRNA and of house keeping genes. A novel and fast method for detection and differentiation has been developed by resolution of protein patterns applying whole cells to MALDI TOF analysis. Measured in the size range of 2,000 to 20,000 Da, the detected proteins indicate the species and classify unknown strains within the genus *Erwinia*. The resulting dendrograms are closely related to those from nucleotide analysis. The method is useful for mass screening and identifies unknown samples by scores of protein relatedness.

Detection and quantification of biocontrol agents

Inmaculada Larena, Paloma Melgarejo..... 359-362

Abstract: This study focuses on the molecular detection and quantification of two biocontrol agents developed in our laboratory: *Penicillium oxalicum* strain 212 (PO212) against soil-borne diseases and *Epicoccum nigrum* isolate 282 (EN282) against aerial diseases. When PCR using a specific primer pair for PO212 and EN282 was combined with serial dilutions of soil or fruit samples on semi-selective media (PoIM and ENSM, respectively) colonies could be rapidly identified and quantified.

Impact of the biocontrol agent *Trichoderma atroviride* SC1 on microbial soil communities of a vineyard

Federica Savazzini, Claudia Maria Oliveira-Longa, Ilaria Pertot 363-367

Abstract: The fungus *Trichoderma atroviride* SC1 is an experimental biocontrol agent that is active against *Armillaria mellea*. We used a highly specific real-time PCR method to monitor the populations of this fungus at different soil depths over several months following its application on the soil surface of a vineyard. The molecular quantification was correlated to colony-forming unit counts. The native communities of bacteria and fungi in the soil were analyzed using automated ribosomal intergenic spacer analysis and transient changes were observed following the application of *T. atroviride* SC1 conidia. Principal component of variance analysis demonstrated that *T. atroviride* SC1 strongly influence the soil microflora in the first two weeks following

inoculation. However, at later dates, environmental conditions had a higher influence on the microbial biodiversity than the application of *T. atroviride* SC1. Soil depth strongly influences the composition and biodiversity of fungal communities.

- Does biocontrol need to consider side effects? Phylloplane chemical changes induced by a BC-yeast preparation and effect on *Cydia pomonella* (Abstract)
Cesare Gessler, Aude Alaphilippe, Sylvie Derridj, Yigal Elad 368
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- Environmental monitoring of *Ceratocystis platani*, agent of the plane canker, by molecular diagnostic in the Lake Geneva region (Abstract)
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- Detection and quantification of *Pseudomonas fluorescens* biocontrol strains by a strain specific real-time PCR approach in the field (Abstract)
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Genomics and transcriptomics in biocontrol

- Complete genome sequencing of *Pantoea agglomerans* strain C9-1
Theo H.M. Smits, Fabio Rezzonico, C. Pelludat, Virginia O. Stockwell, A. Goesmann, J.E. Frey, Brion Duffy 375-378

Abstract: *Pantoea agglomerans* strains are amongst the most effective biocontrol agents for fire blight and other bacterial and fungal plant diseases. Commercialization efforts are hindered however because the species biodiversity includes some strains reported as opportunistic human pathogens. Nonetheless, fire blight biocontrol formulations based on *P. agglomerans* strains C9-1 and Eh325 were registered in the US in 2007, and strain P10c is sold in New Zealand. We sequenced the complete genome of *P. agglomerans* strain C9-1 with ACW funding. We selected this biocontrol strain because of its commercial development, well studied activity and large body of genetic/ ecological data available. The sequencing revealed a 4.88 Mb size, divided over the chromosome (4.025 Mb, currently one gap remaining) and three non-self-transmissible plasmids (0.168, 0.166 and 0.530 Mb, respectively). The preliminary annotation gave a total of 4618 ORFs, and showed the presence of 4 regions containing bacteriophage-related genes and a small genomic island with lower G+C content as the genome backbone. Genes for sugar metabolic pathways found in the genome largely reflect the lifestyle of the organism in a sugar-rich environment. Their presence corresponds to the metabolic spectrum of C9-1 and of the type strain *P. agglomerans* ATCC27155^T, which in fact is a clinical isolate. One of the exceptions may be the presence of two nearly identical sorbitol metabolic gene clusters, located in C9-1 on plasmid pPag2, a substrate which the type strain cannot utilize. Currently, we are assembling the genome sequence of the clinical isolate *P. agglomerans* ATCC27155^T, for comparison with the genome of *P. agglomerans* C9-1. This comparison is expected to reveal the presence of regions important for the biocontrol ability of the latter strain, and for pathogenicity in the clinical isolate. These genotypic features will be tested with other isolates of *P. agglomerans* strains from biocontrol or clinical origin.

- The genomic sequences of the fire blight antagonist *Erwinia tasmaniensis* and *Erwinia billingiae* compared with virulence regions of other *Erwinia* species
Michael Kube, Florian Knaust, Richard Reinhardt, Ina Müller, Klaus Geider 379-382

Abstract: The genomes of *Erwinia tasmaniensis* Et1/99 and *E. billingiae* Eb661 have been sequenced with a chromosome size of 3.8 and 5.1 Mb, respectively. They are antagonistic against *Erwinia amylovora* causing fire blight on rosaceous plants such as apple and pear. Major differences are the ability of *E. tasmaniensis* to induce HR on non-host plants, but lacks EPS synthesis and the ability to metabolize sorbitol. *E. billingiae* produces EPS, metabolizes sorbitol, but lacks HR and sucrose metabolism. *E. tasmaniensis* Et1/99 carries five plasmids from 9 to 45

kb, and Eb661 two large plasmids, which exceed 100 kb. All analyzed *Erwinia* species carry an EPS-encoding region with high homology to the *ams*-cluster of *E. amylovora*. The *hrp/dsp* region of *E. tasmaniensis* is highly related to the corresponding regions of *E. amylovora* and *E. pyrifoliae*, but absent in the draft genome sequence of *E. billingiae*.

Microarray analysis of the induced systemic resistance in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection
Janick Mathys, Miguel F.C. De Bolle, Pieter Timmermans, Bart Lievens, Bruno P.A. Cammue 383-386

Abstract: To meet the current need for biocontrol agentia (BCA) as supplements for pesticides against soil- and especially leaf-specific pathogens, the systemic resistance (ISR), induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 (T382) against *Botrytis cinerea* infection is studied in detail by means of large-scale gene expression analysis using microarray technology, both before and after infection with *B. cinerea*. We opted for T382 because of its proved activity against leaf-specific pathogens, for *B. cinerea* because of its economic relevance on a broad spectrum of host plants and for *A. thaliana* because of the availability of information and techniques. Very little is known about the ISR that is induced by *Trichoderma* spp., especially at the level of gene expression. The few available microarray experiments study the ISR induced by biocontrol bacteria, or the ISR induced by *Trichoderma* spp. examined in the absence of pathogens. Our research results in an accurate and balanced picture of the ISR, induced by T382 in plants challenged with *B. cinerea*. To increase the applicability of our findings, the feasibility of extrapolating the results obtained in *A. thaliana* to tomato is assessed.

Proteomic investigation of resistance to late blight (*Phytophthora infestans*) in potato genotypes treated with acibenzolar-S-methyl
Pier Luigi Burzi, Stefania Galletti, Claudio Cerato 387-389

Abstract: Proteomic analysis could represent an advanced tool to identify proteins associated with disease resistance in plants. This study aimed at highlighting differences in 2-Dimensional Electrophoresis (2-DE) profiles of potato genotypes varying for resistance to late blight, after a treatment by acibenzolar-S-methyl (ASM). This molecule is a well known resistance activator, allowed for organic farming in many countries. In a previous screening among 20 potato genotypes, we found differences for resistance level and response to ASM treatment, measured as variation of two pathogenesis related proteins, chitinase and β -glucanase. In this work we report the preliminary results obtained on selected potato genotypes differing for late blight resistance level. Leaves from 15-days old plants, grown in greenhouse, were treated by ASM, then untreated leaves from the same plants were collected to extract the proteins and perform 2-DE, in comparison to untreated plants. The comparison between the protein profiles of the resistant and the susceptible clones showed 365 spot matching. Three protein spots were only found in the resistant genotype profile. Thirteen spots were down-expressed in the susceptible clone pattern, while 23 spots were up-expressed in the resistant genotype profile. The comparison between ASM treated and untreated plants is under evaluation, to assess whether differences found are constitutive or ASM-related.