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Climate change, biocontrol and integrated plant disease management

Changing management practices and the environment:

impact on soilborne pathogens and biological control

David M. Weller, Timothy C. Paulitz, Dmitri V. Mavrodi, Olga V. Mavrodi,

James A. Parejko, Linda S. Thomashow 3-9

Abstract: Climate change is a regional, national and global environmental policy issue that undoubtedly will be a major economic force for the rest of the 21st century and beyond as nations develop alternatives to traditional fossil fuels to drive and sustain their economic growth. This paper discusses how changes in regional and local environmental conditions, predicted to occur as a result of climate change, may impact crop management practices, diseases and their biocontrol agents. This paper focuses on the Pacific Northwest, located in the Northwest corner of the USA. More specifically, it focuses on wheat production and soilborne diseases in central and eastern Washington State and well-studied *Pseudomonas* spp. that suppress these diseases. What is currently known about the impact of environment and management practices on populations of indigenous *Pseudomonas* spp. that provide natural biocontrol can be used to predict how climate change could impact the biogeography and disease suppressive activity of these biocontrol agents.

Climate change and plant health - increasing importance of biocontrol options for risk management of quarantine pests

Sylvia Blümel 11-14

Abstract: Plant health is directly or indirectly influenced by climatic factors. The potential effects of climate change together with the increasing globalisation of trade with plants and plant products will facilitate the introduction, natural invasion and spread of non-endemic phyto-sanitary plant pathogen species of economic importance to previously unaffected agricultural, horticultural or forestry production areas. Whereas the introduction of quarantine pests can partly be prevented by inspections and monitoring surveys, risk management strategies in case of their establishment and spread under changed climatic conditions are mainly missing. In this context future research needs on climate change effects on plant pathogens and biological control which have been identified are presented and discussed.

Climate change effect on plant – pathogen – beneficial microorganisms interaction in high humidity-promoted tomato diseases

Hananel Ben Kalifa, Dalia Rav David, Menahem Borenshtein, Ran Shulchany,

Yigal Elad 15-18

Abstract: Climate change refers to changes in the means and/or variabilities of climate parameters. Plant pathogens have a range of environmental conditions which allows them to survive and cause disease. Environmental changes might cause alterations in distribution, survival and plant-pathogen interactions; these changes can increase or decrease epidemical

events. The suppressive effect of beneficial organisms may also be affected by environmental conditions. Two humidity promoted polycyclic diseases were studied: i. late blight (*Phytophthora infestans*) – a fast developing and destructive disease in tomato and potato plants. A critical factor for disease epidemic occurrence is 6-8 hours of wetness which allows penetration into the plant tissue; ii. grey mould (*Botrytis cinerea*) – infects many crops including tomato and proliferates at high humidity, few hours of wetness are a prerequisite for infection. Tomato plants inoculated with *P. infestans* with wetness duration of 4, 6, 8, 12 and 24 hours showed decrease in disease frequency and severity under 8 and 12h wetness as compared with 24h. *B. cinerea* infection severity was different between 4 and 10h wetness duration. Spraying the plants with two yeast, three bacterium isolates and *Trichoderma* followed inoculation with *P. infestans* under wetness durations of 8 and 24h, revealed an effect of wetness duration on biocontrol activity; 24h wetness duration less disease control was observed. When treating with the microorganisma at pre- and post-inoculation, the same disease suppression was obtained for 8 and 24h wetting periods. Two of the BCAs decrease sporangia formation. No interaction in grey mould suppression was observed between biocontrol agents and wetness duration. It seems that environmental conditions which decrease late blight intensity allow better activity of the introduced biocontrol agents. Climate change is expected to affect rain quantity and frequency and as a result the duration of wetness may be affected. This in turn, in some cases as demonstrated in the present work, may affect the ability of beneficial organisms to suppress diseases.

Is the climate really changing in favour of biopesticides?

Willem Ravensberg 19-22

Abstract: Many factors influence the adoption of biopesticides in the marketplace. Some are hampering biopesticide, other favour them. Changes in legislation, and in political, cultural and social perceptions determine the demand for sustainable crop protection agents. Research and technological discoveries create new possibilities for development of better products. Globalisation opens new markets and offers potential usages, but also creates more problems such as invasive pests and diseases. Trends in the biopesticide industry stimulate new products and markets. The current macro-environmental trends support the assumption that demand for alternative crop protection products will grow rapidly to replace conventional chemical pesticides.

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Climate change, biocontrol and integrated plant disease management: Problems and perspectives of biocontrol under Mediterranean conditions

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Antagonistic endophytes from mistletoes as bio-resource to control plant as well as clean room pathogens <i>Gabriele Berg, Kathrin Hartenberger, Stefan Liebming, Christin Zachow</i>	29-32
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Abstract: *Viscum album* is a hemiparasitic shrub on a wide range of wood species. The bacterial and fungal communities of *Viscum album* subsp. *album* and three different host species (*Malus domestica*, *Juglans nigra* and *Acer tataricum*) were analysed by cultivation dependent methods. Endophytes were isolated from leaves and seeds of *Viscum album* as well as from branches of the host trees on five different growing media. The isolated endophytes were screened for antagonistic effects i) against plant pathogens (*Alternaria alternata*, *Botrytis cinerea*, *Phytophthora infestans*, and *Sclerotinia sclerotiorum*), and ii) against clean room inhabitants or pathogens (gram- positive bacteria: *Staphylococcus epidermidis*, *Propionobacterium acnes*, *Paenibacillus polymyxa*, *Geobacillus stearothermophilus*, *Bacillus pumilus*; gram-negative bacteria: *Stenotrophomonas maltophilia*, *Escherichia coli*, *Pseudomonas aeruginosa*; fungi: *Verticillium dahliae*, *Aspergillus niger*, *Candida albicans*) by dual culture assay. Beside the high proportion of antagonistic isolates against both groups in general, we found similarities but also clear differences between parasite and host. Mistletoes, especially the seeds, contained a higher antagonistic potential than the host plants. Plant-associated endophytic microorganisms from parasitic plants are an interesting bio-resource to control plant pathogens but also clean room inhabitants/pathogens.

New bio-resources and techniques for biocontrol

Screening criteria for the development of commercial products for biocontrol of plant pathogens <i>Jürgen Köhl, Bernard Blum, Philippe Nicot, Michelina Ruocco</i>	35-37
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Abstract: Antagonists for use in commercial biocontrol products have to fulfil many different requirements. Besides being active control agents against the specific targeted plant pathogens, they must be safe and cost effective. The development of new biocontrol products starts with screening programs including hundreds or thousands of candidates. For commercial use, important criteria are market size, efficacy, ecological characteristics, production costs, safety, environmental risks and protection of intellectual property rights. A stepwise screening considering these very different aspects is proposed.

The use of molecular tools to unravel the complex interaction of biological control strains, pathogens and indigenous microbial communities <i>Simone Dealtry, Guo-Chun Ding, Nicole Weinert, Yvette Piceno, Gary L. Andersen, Michael Schloter, Gabriele Berg, Leda Mendonça-Hagler, Rita Grosch, Kornelia Smalla</i>	38
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Endophytes of grapevine as potential control agents against fungal vine diseases <i>Martin Kirchmair, Sabine Trenkwalder, Lars Huber, Sigrid Neuhauser</i>	39-43
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Abstract: Grapevine trunk diseases like esca are difficult to control and cause significant economic loss in vine growing areas all over the world. The causal organisms are xylem inhabiting, pathogenic fungi sharing habitat with other endophytic microorganisms. Xylem sap of

twigs of healthy and esca affected vines was plated onto nutrient media. The isolated endophytes were identified morphologically and by DNA sequence analysis. Dominant yeast endophytes were *Aureobasidium pullulans*, *Cryptococcus magnus* and *Rhodotorula cf. glutinis*. The most abundant endophytic bacteria belonged to the genera *Curtobacterium*, *Frigoribacterium*, *Pantoea*, *Pseudomonas* and *Bacillus*. The antagonistic potential of selected endophytes against grapevine pathogenic fungi (*Phomopsis viticola*, *Phaeoconiella chlamydospora*, *Cylindrocarpon* sp., *Roesleria subterranea*) was evaluated in vitro. Isolated yeasts (e.g. *Aureobasidium pullulans*) and bacteria (esp. Gammaproteobacteria) considerably inhibited the growth of grapevine pathogenic fungi. To test the effect on plant growth cress tests with the culture-supernatant of endophytic yeasts cultures were conducted. *Sporobolomyces roseus*, *Rhodotorula pinicola* and an unknown *Cryptococcus* species promoted the root growth of cress seedlings. These results point towards endophytes playing a critical role as plant growth promoters and in the defense against plant pathogens. Endophytes have the potential to play a future key role as biocontrol agents and biofertilizers.

Biological control of *Rhizoctonia solani* by stimulation of naturally present antagonistic *Lysobacter* spp.

Joeke Postma, Mirjam Schilder, Els Nijhuis 45-47

Abstract: Disease suppressive soils towards *Rhizoctonia solani* AG2 were found to comprise three closely related antagonistic species, i.e. *Lysobacter antibioticus*, *L. capsici*, and *L. gummosus*. Isolates of these species showed strong *in vitro* inhibition of several plant pathogens. The current research focuses on the ecology of these *Lysobacter* species with the aim to stimulate their natural population in soil and as a consequence enhance disease suppressiveness of soil systems. Recent results showed that chitin, chitosan, yeast cells and mushroom powder stimulated *Rhizoctonia* disease suppression in a bioassay with sugar beet. Meanwhile, *Lysobacter* populations detected by QPCR were about 5 to 20 times higher than in the control soil. Other organic compounds such as compost or cellulose were not effective. With these results we finally aim to develop a more sustainable growing system, benefitting from the microflora naturally present in soil.

Biocontrol of rice sheath blight by *Trichoderma*: selection of promising BCAs and development of strain specific monitoring tools

Shahram Naeimi, Sándor Kocsubé, Zsuzsanna Antal, Seyed Mahmood Okhovvat, Mohammad Javan-Nikkhah, Csaba Vágvölgyi, László Kredics 49-53

Abstract: A total of 202 *Trichoderma* strains were isolated from the soil and rice phyllosphere in paddy fields at 45 locations throughout the Mazandaran province, Northern Iran. *T. harzianum* and *T. virens* proved to be the most frequent species in these habitats. Based on the results of *in vitro* antagonism tests and glasshouse trials, strains with biocontrol potential against the rice sheath blight pathogen *Rhizoctonia solani* were selected. UP-PCR based SCAR markers were developed for the specific detection and monitoring of *T. harzianum* AS12-2, the most promising biocontrol strain.

Effect of nutrient amendment and osmoadaptation in blossom colonization of pome fruit trees and efficacy of biocontrol of *Erwinia amylovora*

Jordi Cabrefiga, Anna Bonaterra, Jesus Frances and Emilio Montesinos 55-57

Abstract: The efficacy of *Pseudomonas fluorescens* EPS62e in the biocontrol of *Erwinia amylovora*, the causal agent of fire blight disease, depends on the colonization of plant surfaces. A procedure to increase cell survival in the phyllosphere was developed consisting of nutrient amendment, osmoadaptation and their combination. Glycine and Tween80 were selected as appropriate substrates to add as nutrient complements in the formulation of EPS62e, both nutrients are used by EPS62e and not by *E. amylovora* strains. Colonization and survival of EPS62e on detached flowers studies were performed in controlled environmental conditions. At high RH population levels of EPS62e remains stable and were not affected by the treatments. Whereas at low RH, population levels of EPS62e fall down and an enhancement of their survival

in the nutrient amended and osmoadapted was observed. Population dynamics of EPS62e in field conditions were assessed by real-time PCR and CFU-counting methods. Values obtained by both methods were quite correlated and population levels of EPS62e decreased to steady-state and reach values around 10^6 CFU/blossom in the standard application and were significantly higher, around 10^7 CFU/blossom, in nutrient amended also in osmoadapted treatment and especially in the combined treatment. Finally, the efficacy in the control of fire blight infections under field conditions was also assessed and the best treatment was the combination of nutrient amendment with the osmoadaptation.

Molecular mechanisms and signaling in biocontrol

Cell-to-cell communication controls biocontrol activities in members of the *Burkholderia cepacia* complex (Bcc)

Silvia Schmidt, Judith F. Blom, Jakob Perntaler, Gabriele Berg, Eshwar Mahenthalingam, Leo Eberl 61

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Novel approaches for biocontrol of crown gall disease

Natalia Dandurishvili, Natela Toklikishvili, Naili Giorgobiani, Marina Tediashvili, Marianna Ovadis, Inessa Khmel, Erno Szegedi, Alexander Vainstein, Leonid Chernin 62

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Distribution of antimicrobial peptide biosynthetic gene markers in plant-associated *Bacillus* spp. and relationships with antagonistic activity against bacterial plant pathogens

Isabel Mora, Jordi Cabrefiga, Emilio Montesinos 63-66

Abstract: A large collection of *Bacillus* isolates obtained from natural samples, including aerial plant parts, rhizosphere and bare soil in agricultural and land environments from the North-eastern part of Spain, were characterized for the presence of the antimicrobial peptide biosynthetic genes *srfAA* (surfactin), *bacA* (bacylloisins), *fenD* (fengycin), *bmyB* (bacylloisins), *spaS* (subtilin) and *ituC* (iturin). Most isolates had at least one of the biosynthetic genes and presented 2-to-4 genes simultaneously. The most frequent genotypes were *srfAA bacA bmyB* (15.2%) and *srfAA bacA bmyB fenD* (14.1%). The analysis of *in vitro* antagonism against eight species of plant-pathogenic bacteria indicated that the proportion of highly active antibacterial strains increased with the number of simultaneous genes per strain.

Evaluation of anti-phytoplasma properties of surfactin derived from Iranian native *Bacillus subtilis* isolates using Real Time PCR

Najmeh Askari, Gholamreza Salehi Jouzani, Matin Mohammadi Pour, Maryam Mousivand, Saeed Abbasalizadeh, Ali Hagh Nazari 67-71

Abstract: In the present study, the effect of surfactin derived from Iranian native *Bacillus subtilis* isolates has been investigated against phytoplasma Candidatus "Phytoplasma aurantifolia" agent of lime witches broom disease (WBDL) which causes a considerable loss in lime production in Iran and other countries in the region of Persian Gulf. For this purpose, eight surfactin producing isolates were evaluated for quantity of surfactin production by HPLC. Quantitative analysis by HPLC showed that one strain had the highest surfactin production (650mg/l) and were selected for surfactin extraction. After optimization of surfactin extraction, it was injected into witches broom phytoplasma-infected seedlings of lime by using syringe injection. To compare tetracycline effect with surfactin effect against phytoplasma activity and also to investigate probable synergistic effect of them, we planned to also treat plants with both mentioned antibiotics at the same time. Two specific primers pairs were designed for qualitative and quantitative detection of phytoplasma in infected plants. Real Time PCR conjugated with

Fluorescent SYBR® Green I dye and absolute quantification has been developed for rapid, sensitive and quantitative analysis to assess surfactin bioactivity against phytoplasma, and also to determine concentration of phytoplasma in infected seedlings before and after treatment. The results showed that the combination of surfactin had caused significant reduction of phytoplasma population in infected plants, and also the combination of surfactin and tetracycline had the highest toxicity against phytoplasma.

Modulation of the lipopeptide pattern secreted by *Bacillus subtilis*
upon colonization of different plant roots

Marc Ongena, Hélène Cawoy, Maïté Smargiassi, Venant Nihorimbere,

Emmanuel Jourdan, Philippe Thonart 73-77

Abstract: A lot of environmental factors may modulate the production of antibiotics by plant beneficial rhizobacteria in general and of lipopeptides by *Bacillus* in particular. Direct evidence for an ecological role through the demonstration of an efficient lipopeptide production in situ is thus requested. This was the first aim of this study achieved by using an optimized HPLC-coupled electrospray ionization MS that also allowed to compare the cLP profiles secreted by strain S499 in the rhizosphere of different plants. Analyses of these root extracts revealed both quantitative and qualitative modifications in the lipopeptide signature, suggesting that the root exudate content may influence the synthesis of these molecules in a plant-specific manner. The possible impact of such variations in the cLP profile on the biocontrol potential of the producing strain is discussed.

Insecticidal activity in root-associated, plant-beneficial pseudomonads

Monika Maurhofer, Beat Ruffner, Maria Péchy-Tarr, Christoph Keel 78

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From 'omics' to the field: functional genomics and implementation models
of *Trichoderma* BCAs

Sheridan L. Woo, Michelina Ruocco, Francesco Vinale, Roberta Marra,

Stefania Lanzuise, Felice Scala, Matteo Lorito 79

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Volatile secondary metabolites of the biocontrol fungus *Trichoderma atroviride*:
Profiling by HS-SPME-GC-MS

Norbert Stoppacher, Bernhard Kluger, Susanne Zeilinger, Rudolf Krska,

Rainer Schuhmacher 80

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Role of G protein signalling in host sensing of the mycoparasite *Trichoderma atroviride*

Markus Omann, Sylvia Lehner, Carolina Escobar Rodriguez,

Norbert Stoppacher and Susanne Zeilinger 81-82

Abstract: Fungi of the genus *Trichoderma* are frequently found in soil and associated with dead organic material but are also able to interact with plants, animals and other fungi. Due to these manifold lifestyles, a broad spectrum of environmental signals has to be recognized by *Trichoderma* and integrated into respective signal transduction pathways. Recent studies revealed the involvement of G protein signaling in governing processes relevant for the mycoparasitic interaction of *T. atroviride* with other fungi and both the Tga3G subunit as well as the Gpr1 G protein-coupled receptor were shown to be relevant for recognizing the host fungus.

Identification of *Ampelomyces quisqualis* genes involved in the early stage
of mycoparasitism (host recognition) of powdery mildew

Lorenzo Tosi, Massimo Delledonne, Alberto Ferrarini, Cesare Gessler,

Monika Maurhofer, Ilaria Pertot 83

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Biocontrol agents: Ways to commercialization

How to deliver beneficial microbes <i>Jana Monk, Emily Gerard, Sandra Young, Keith Widdup, Trevor Jackson, Maureen O'Callaghan</i>	87
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Practical use of beneficial rhizosphere bacteria in crop production <i>Jolanta J. Levenfors, Jamshid Fatehi, Margareta Hökeberg, Mariann Wikström, Ann-Sofie Birch-Jensen, Christopher J. Welch</i>	88
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Abstract: In frame of a Chinese German collaboration project we have performed extended greenhouse and field trials in several Chinese regions, different in their climate and soil conditions, to investigate the beneficial effect on crops and vegetables exerted by *Bacillus*-based bioformulations. Distinct positive effects were obtained in treatment of maize, potatoes, vegetables, and ornamentals; however time and dosage of the applied bacterial spores did affect success of the treatment. Whilst “coating” of maize seeds and potato tubers ruled out as a useful method, vegetables and cut flowers were most successfully treated during transplanting. The success of the treatment did depend also from an appropriate concentration of the applied bacteria. Too high concentrations are useless or harmful. In general, we recommend use of appropriately diluted *Bacillus* formulations containing 2.5 to 5×10^{13} bacterial spores (corresponding to 1 to 2l Rhizovital[®]) per ha.

All over the plant - Microbial products for enhanced health, fitness and quality <i>Henry Müller, Markus Verginer, Christin Zachow, Gabriele Berg</i>	94
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Review of factors influencing the success or failure of biocontrol: technical, industrial and socio-economic perspectives <i>Philippe C. Nicot, Claude Alabouvette, Marc Bardin, Bernard Blum, Jürgen Köhl, Michelina Ruocco</i>	95-98
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Abstract: Although their role in plant health management is still quite modest, ever increasing expectations are placed on biocontrol agents for the complementation or replacement of pesticide use. Despite decades of research and more than 12,000 scientific papers on biological control of plant diseases, little more than 14 microbe-based commercial products are presently registered for use against diseases in the European Union. Through a review of published scientific literature, economic and market analyses and a survey of 675 farmers in 9 European countries, difficulties and conditions for success have been identified. The most salient features and future outlook are presented in terms of scientific/technical aspects, production costs and return on investment as well as (somewhat unexpected) factors deemed to be most influential on the evolution of the biocontrol market in the coming decades.

Registration of plant protection products containing micro-organisms in the EU – changes through the new Regulation 1107/2009

Jacqueline Süß, Rüdiger Hauschild 99-102

Abstract: The legal framework and data requirements for the registration of biological plant protection products in the EU are regulated in Council Directive 91/414. By June 14, 2011 the Regulation 1107/2009 will apply. The current situation will be presented with an interpretation of the data requirements and experiences from the regulatory procedure in the EU and different EU member states. An overview about changes imposed by Regulation (EC) 1107/2009 will be given with regard to consequences for producers of biological plant protection products.

Mass production of ecologically competent *Metarhizium anisopliae* spores for pest management

Verena Niedermayr, Hermann Strasser 103-106

Abstract: As new insect pests invade Europe (e.g. *Diabrotica* or the Siberian grasshopper) and arthropods, such as FSME- or *Borrelia*- infested ticks, are to be antagonised, the BIPESCO Team Innsbruck focused its interest in the product development of fungal biocontrol agents, which can be applied as foliar spray.

The objective of our study was to compare simple solid state mass production systems for high yield *Metarhizium anisopliae* spore production. Methods and tools are syntonized to the used *Metarhizium* production strain BIPESCO 5. Mineral and organic carriers are compared in solid state fermenter systems to enable the production of improved high concentrate spore formulations. First data on expanded open pored clay granules are presented, which preferentially stimulates fungal sporulation and spore production after soaking in Sabouraud Dextrose medium. Grinding of the overgrown mineral carrier showed either no negative effect on the spore vitality and efficacy, nor was the formulation contaminated with organic impurities.

Bacteria controlling cucumber foot and root rot and promoting growth of cucumber and tomato in salinated soils

Dilfuza Egamberdieva, Gabriele Berg, Vladimir Chebotar, Igor Tikhonovich, Faina Kamilova, Shamil Z. Validov, Ben Lugtenberg 107-112

Abstract: Seventeen percent of cucumber plants grown in a Uzbek greenhouse were diseased. The major cucumber and tomato pathogens of Uzbek agricultural soils were identified as strains of *Fusarium solani*. Fifty two beneficial bacteria from collections of our institutes were screened for their ability to promote growth and/or to control diseases caused by *F. solani* on cucumber and tomato plants. The five best strains were used in large scale greenhouse trials. Four out of five strains significantly controlled cucumber foot and root rot, reducing the percentage of diseased plants from 54% in the negative control to between 10 and 29% in bacterized plants. All five strains increased the dry weight, by 29 up to 62%. In two consecutive years all five strains significantly increased the plant height (by 4 to 15%) as well as the fruit yield (by 12 to 32%). Tests of plant-beneficial traits suggest that auxin production, antibiosis and competition for nutrients and niches are mechanisms involved in the observed plant growth stimulation and biocontrol. The results with tomato were similar. We conclude that many beneficial bacteria isolated from plants grown on non-salinated soil are perfectly able to promote plant growth and control plant diseases in salinated soil. In other words, salination caused by a possible future climate change does not seem to be a threat for the application of presently used plant-beneficial bacteria. In addition, our results show that the dogma that beneficial strains should be isolated from the plant and climate on/in which they should be applied is not valid: all our strains were isolated from plants other than cucumber and came from cold or moderate climates.

Effect of fungal and bacterial bio-control strains and *Rhizoctonia solani* on indigenous microbial community in the lettuce rhizosphere

Rita Grosch, Simone Dealtry, Gabriele Berg, Leda Mendonça-Hagler, Kornelia Smalla 113

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- Impact of a microbial consortium on the native *Pseudomonas* and mycorrhizal community in the rhizosphere of nine different maize genotypes
Andreas von Felten, Carolin Schwer, Olivier Couillerot, Yvan Moënne-Loccoz, Jan Jansa, Geneviève Défago, Monika Maurhofer 114
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- Genetic structure and dynamics of the oomycete and fungal communities colonizing the rhizosphere and the effluents of hydroponic tomato plants after the introduction of the biocontrol agent *Pythium oligandrum*
Jessica Vallance, Franck Déniel, Georges Barbier, Patrice Rey 115-118

Abstract: In hydroponic cultures, the recycling of drainage water is the main source of introduction and dissemination of pathogens. The spreading of the biocontrol agent *Pythium oligandrum* was investigated in relation to the recirculation of the nutrient solution. The structure and dynamics of the fungal communities colonizing the effluents and the rhizosphere of hydroponic tomato plants were also assessed. Real-time PCR and plate counting demonstrated the persistence of large amounts of the antagonistic oomycete in the rhizosphere throughout the cropping season (April to September). Despite its abundance on roots, no traces of *P. oligandrum* were detected in the different effluents of the soilless system investigated. *P. dissotocum* (ubiquitous tomato root minor pathogen) colonized the rhizosphere and the effluents only in summer. There was a slight reduction of *P. dissotocum* populations in *P. oligandrum*-inoculated root systems. Single-Strand Conformation Polymorphism (SSCP) analyses revealed that the genetic structure of the fungal communities colonizing the rhizosphere and the effluents was different and evolved throughout the cropping season. This temporal evolution was independent from the inoculation and the persistence of the antagonistic oomycete *P. oligandrum*.

- The effect of *Fusarium oxysporum* f. sp. *lycopersici* and the arbuscular mycorrhizal fungus *Glomus mosseae* on tomato (*Solanum lycopersicum* L.) intercropped with cucumber (*Cucumis sativus* L.)
Karin Hage-Ahmed, Vladimir Chobot, Andreas Voglgruber, Franz Hadacek, Siegrid Steinkellner 119-122

Abstract: The rhizosphere is known to be a highly dynamic environment, where plant – microbe, plant – plant and microbe – microbe interactions take place. Components of root exudates can act as trigger for such interactions. For this work a model system was created consisting of tomato (*Solanum lycopersicum* L.), the intercropping partner cucumber (*Cucumis sativus* L.), the soilborne tomato pathogen *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and the arbuscular mycorrhizal fungus *Glomus mosseae* (GM).

Tomato/tomato and tomato/cucumber combinations were grown together in pots and received the following treatments: FOL, AMF, FOL and AMF in combination, to assess changes in root exudation, the AMF colonization rate and the FOL infection rate. Furthermore, the exudates were used for in vitro tests of the initial behaviour and development of FOL and for analysing the components (e.g. sugars, secondary metabolites) of the exudates. FOL significantly increased the AMF colonization rate in the FOL and AMF combination compared to the AMF treatment in tomato/cucumber, whereas for the tomato/tomato combination no such effect could be detected.

Short overview about new biocontrol studies

- Biology of black rot of grapevine (*Guignardia bidwellii*) and approaches towards its control by non-chemical methods
Eckhard Koch, Cornelia I. Ullrich, Bernd Loskill, Michael Maixner, Daniel Molitor, Beate Berkelmann-Löhnertz 125
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Control of <i>Plasmodiophora brassicae</i> – literature review and future prospects <i>Sigrid Neuhauser, Martin Kirchmair</i>	127-130
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Abstract: *Plasmodiophora brassicae* Woronin (Plasmodiophorida) is the casual agent of clubroot, the most important soil-borne disease of cruciferous crops worldwide. Losses were estimated 10-15% of the annual harvest combining all cruciferous crops including important food-, industrial- and energy-plants like cole crops, oil seed rape or turnips. The soil-borne and plant-associated nature of plasmodiophorids as well as their obligate biotrophic multi-stage life cycle with multiple zoosporic, plasmodial, and resting stages has hampered research on this group of pathogens. No efficient control strategies are available so far and resistance of most cultivars has been proven elusive. Different attempts of biological control including soil-borne and endophytic microorganisms were tested with changing success. A number of potential biocontrol agents were successfully tested in green house tests, but in field trials the efficacy of these biocontrol agents varied considerably. Soil properties play a key role for the infection process and disease development as well as for the success of biological control. Therefore, until now best results to reduce disease symptoms were obtained with integrated pest management strategies. One reason why biocontrol agents were not successfully applied until today can be that the Plasmodiophorida are taxonomically and ecologically far different from “true fungi” or most other soilborn pathogens. Recent molecular phylogenies place them in the eukaryotic supergroup “Rhizaria” with a close affiliation to the Cercozoa and Foraminifera. Strategies successful in controlling fungal diseases cannot necessarily be transferred to organisms of a very different taxonomic affiliation. Increased knowledge about the taxonomic position and the biology of this soil-borne pathogen offers potential targets for the future control of the clubroot pathogen.

Powdery mildew on cereals – an increasing problem in triticale cultures <i>Caterina L. Matasci, Stefan Kellenberger, Fabio Mascher</i>	131-134
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Abstract: Powdery mildew, caused by the obligate biotrophic ascomycete *Blumeria graminis* DC Speer can reduce yield up to 40% without control measures. The first report of the disease on triticale (*x Triticosecale* Wittmack) in Switzerland took place in 2001. Since 2005, which marked the year of the first major epidemic, *B. graminis* has become established and rapidly adapts to an increasing number of triticale cultivars. This situation is observed in different European triticale growing countries. In a situation where the climate change is modifying the national and international ranges of pests and diseases and where a strong increase in the demand for cereals is predicted for the future, biological control should focus on selection for resistant cultivars in combination with adequate culture techniques and, if possible, the use of antagonistic organisms.

Evaluation of naturally occurring <i>Pseudomonas</i> spp. and a biopesticide based on <i>Trichoderma koningiopsis</i> as potential biological control agents of <i>Olpidium virulentus</i> in Fique <i>Alba Marina Cotes, Leonardo Sastoque, Lina Rada, Camilo Beltrán Acosta, Carolina González, Daniel Osorio, María C. Cepero de Garcia</i>	135-139
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Abstract: We report the presence of *Olpidium virulentus* in roots of fique plants where Macana virus disease has been detected. When fique plants obtained *in vitro* or lettuce seedlings used as model plant were inoculated with resting spores of *O. virulentus*, severe disease symptoms and dramatic fresh weight reduction were expressed. With the aim to select potential biological control agents, sixteen indigenous isolates corresponding to *Pseudomonas* spp. and a biopesticide based on *T. koningiopsis* were evaluated on lettuce planted in soil infested with *O. virulentus*. Six isolates of *Pseudomonas* spp. and the biopesticide based on *T. koningiopsis* reduced of both the

number of *Olpidium* sp. resting spores in roots, and the incidence and severity of the disease. These microorganisms also improved germination, promoted plant growth, presented solubilization phosphatase activity and produced indol acetic acid (IAA).

- Control of downy mildew on cucumber (*Pseudoperonospora cubensis*)
by plant extracts and a bacterial preparation
*Christina Schuster, Andrea Nowak, Peggy Marx, Ute Gärber,
Stavroula Konstantinidou-Doltsinis, Barrie Seddon, Annegret Schmitt* 140
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- Biochar impact on plant development and disease resistance in pot trials
*Yael Meller Harel, Max Kolton, Yigal Elad, Dalia Rav-David,
Eddie Cytryn, Menahem Borenshtein, Ran Shulchani, Ellen R. Graber* 141-147
Abstract: Biochar (charcoal) is the solid co-product of biomass pyrolysis (thermal decomposition in the absence of oxygen). Amendment of soil with biochar is known to improve soil tilth, nutrient retention and crop productivity. We studied the effect of soil-applied biochar on plant productivity and plant foliar diseases. Biochar amendments to sand at levels of 1-3 weight % enhanced the growth of tomato and pepper plants. In addition, suppression of the powdery mildew caused by *Podosphaera aphanis* on strawberry plants grown in commercial coconut fiber:tuff growing mix was observed. Resistance was maximal with 3% biochar amendment as long as 6 months following planting. Reduction rates were 20 to 93% depending on the biochar percentage and sampling date. However, gray mold caused by *Botrytis cinerea* on strawberry leaves was not reduced upon biochar treatment. Similarly, root-nematode *Meloidogyne javanica* was not affected by biochar amendments in tomato grown in sandy soil. Biochar amendments moderately enhanced abundances of culturable general bacteria and *Bacillus* spp. but had no apparent effect on other tested culturable microorganisms. Studies designed to clarify the important systemic resistance metabolic pathways and its elicitors are underway. Biochar in soil has a very slow turnover (half-life of 1000s of years), and therefore, when introduced to soil it results in quasi-permanent sequestration of carbon. Soil treatment with biochar can thus serve as a climate change mitigation tool and an agriculture adaptation tool.

- Acidic pH supports efficient antagonist-based control
of the bacterial plant disease fire blight
Doris Pester, Ulrike Persen 149
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- Cooperating bacterial and fungal biocontrol strains with beneficial interactions
in sugar beet rhizosphere
*Christin Zachow, Henry Müller, Ralf Tilcher, Jamshid Fatehi,
Massimiliano Cardinale, Gabriele Berg* 150
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- Influence of temperature on morphology and physiology of different isolates
of *Ampelomyces quisqualis*
*Dario Angeli, Monika Maurhofer, Susanna Micheli, Cesare Gessler,
Ilaria Pertot* 153-157
Abstract: Temperatures significantly influenced the growth rate of the colonies and the highest radial growth rate was measured at 20°C for all strains. Interestingly, original climate at which *A. quisqualis* was isolated did not influence an adaptation to the temperature. In fact, strains isolated in countries with a warm climate grew well at low temperature and vice versa. Observing

the conidial germination phase, tube length and especially germination rate of *A. quisqualis* conidia increased greatly in the presence of powdery mildew conidia in different temperature. Temperature in the tested range had no influence on the germination rate, but seems to have an effect on tube length. These results suggest that different strains of *A. quisqualis* may react in a different way to temperature and this aspect should be considered in the selection of commercial strains, especially in a view of climate change.

Screening of new *Trichoderma* spp. isolates

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Selecting highly effective strains of *Ampelomyces quisqualis* for the control of powdery mildews

Dario Angeli, Monika Maurhofer, Cesare Gessler, Ilaria Pertot 159-163

Abstract: Genetically different *Ampelomyces quisqualis* strains isolated from different locations, fungal hosts and host plants were selected; pathogenicity and aggressiveness on different powdery mildew species were evaluated. Under controlled conditions all the selected strains reduced the sporulation of powdery mildews (*Podosphaera xanthii*, *Podosphaera aphanis*), but with a different level of reduction among strains. Strains can be divided in highly and poorly aggressive, and their behavior is similar on the studied powdery mildews. The susceptibility versus *A. quisqualis* varies among the different tested powdery mildews. Results suggest that there are not adaptations of *A. quisqualis* to powdery mildew species from whatever was the original host specie the mycoparasite strain was isolated as no differential interaction was observed, independently of their phylogenetic groups or geographical origin. Some of the tested strains could be appropriate candidates for future commercial development in environmental conditions to control different powdery mildew species.

Microbial diversity and populations-dynamics of the wheat phyllosphere analysed by denaturing gradient gel electrophoresis (DGGE)

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Endophytic and epiphytic bacteria associated with *Sphagnum* mosses as perspective objects for agricultural biotechnology

Shcherbakov, A. V., Krikovtseva, A. V., Kuzmina, E. Yu., Berg, C., Malfanova, N. V., Cardinale, M., Berg, G., Chebotar, V. K., Tikhonovich, I. A. ... 165-171

Abstract: The aim of this study was to isolate promising bacterial strains associated with *Sphagnum* mosses possessing beneficial properties for agricultural crops as perspective objects for agricultural biotechnology. Previously it was shown that *Sphagnum* mosses are associated with unique microorganisms which have important functions for *Sphagnum* mosses and for the bog ecosystem as a whole. Study was focused on sampling of two *Sphagnum* species from bogs located at different geographical areas of the Kolskyi peninsula (Russia) and Alps mountains (Austria) which plays a different ecological role in the ecosystem of bogs. About 150 strains of bacteria were isolated from the tissues of *Sphagnum* plants, their culture and morphology properties have been investigated. Antagonistic properties of isolated strains against a number of phytopathogenic fungi and bacteria have been studied. It was shown that more than 60% of all isolates demonstrated strong antifungal properties (*in vitro*). Strains capable of promoting plant-growth, grow on the nitrogen-free medium and solubilize of not soluble phosphates were selected. Selected strains can be used for further study in greenhouse experiments with agricultural crops.

- Study of interaction between toxigenic fungi and endophytic bacteria in the wheat grain for development of the methods for control of toxigenic infections and production of high quality foodstuffs
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- Priming – an eco-friendly strategy to improve abiotic stress tolerance of Oilseed rape (*Brassica napus*)
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- A new cellulase producing species *Trichoderma parareesei* nom. prov. reveals high antagonistic potential against soil and plant pathogenic fungi
Lea Atanasova, Walter M. Jaklitsch, Monika Komon-Zelazowska, Christian P. Kubicek, Irina S. Druzhinina 174
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- The induction of resistance in inflorescences of *Vitis vinifera* L. by *Burkholderia phytofirmans* strain PsJN against *Botrytis cinerea* Pers.
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- Field studies of epidemiological development of *Fusarium* sp. in wheat during growing period and in harvested crops with molecular methods
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- Biological control of root rot caused by *Pythium aphanidermatum* and growth promotion in hydroponic cucumber with microorganisms from mangrove
Élida B. Corrêa, José Abrahão H. Galvão, Wagner Bettiol 177-181
Abstract: The prospection of biological control agents in similar environments to the microbe application improves the chances of microorganisms establishment added to the environment. The low survival of these beneficial microorganisms added to hydroponic environment is a problem for the growth promotion and root rot biological control success in hydroponic crops. Because of the environmental similarity between hydroponic systems and mangrove ecosystems, the aim of this work was to evaluate the ability of mangrove microbes to control root rot caused by *Pythium aphanidermatum* and to improve plant growth in hydroponic cucumbers. Among the 28 strains evaluated for disease control in small-hydroponic system using cucumber seedlings, *Gordonia rubripertincta* SO-3B-2 alone or in combination with *Pseudomonas stutzeri* (MB-P3A-49, MB-P3-C68 and SO-3L-3), and *Bacillus cereus* AVIC-3-6 increased the seedlings survival and were subsequently evaluated in hydroponic cucumbers in a greenhouse. *Bacillus cereus* AVIC-3-6 protected the plants from stunting caused by the pathogen and *Gordonia rubripertincta* SO-3B-2 and *Pseudomonas stutzeri* MB-P3A-49 increased the plant growth. We concluded that microorganisms from mangroves are useful as biocontrol agents and for improving plant growth in hydroponic crops.
- Strains of *Coniothyrium minitans* reduce the emission of apothecia of *Sclerotinia sclerotiorum*
Marcelo A. B. Morandi, Carlos E. O. Silva, Lúcio B. Costa, Wagner Bettiol 183-187
Abstract: Sclerotia of *Sclerotinia sclerotiorum* (*Ss*) can survive for long time in soil and are the main inoculum source of the white mold disease. An alternative for reducing this inoculum is the use of parasites, such as *Coniothyrium minitans* (*Cm*). We evaluated the potential of *Cm* isolates for the biological control of *Ss* in beans. The effect of the temperature on the growth of 15

isolated of *Cm* was evaluated *in vitro*. The hyperparasitism ability of *Cm* was evaluated in soil infested with sclerotia and conditioned in pots. The infested soil was treated with conidia suspension of the antagonists, fluazinan or sterile distilled water. After seven days at 20°C, the sclerotia were removed from soil and placed inside Petri dishes over bean leaves previously disinfested. The germination and parasitism of sclerotia were evaluated after 7 to 10 days. To evaluate the apothecia emission, soil infested with sclerotia of *Ss* and treated as described was maintained at 18°C and the number of emerged apothecia was counted up to 84 days after inoculation. The emergence of bean plants in soil infested with sclerotia and mycelium of the pathogen and treated as described was evaluated in greenhouse. The ideal temperature for growth of *Cm* isolates varied from 18 to 19°C and at 30-35°C they were completely inhibited. The isolates of *Cm* promoted less than 10% of reduction in viability of the sclerotia, but they significantly reduced the emission of apothecia. Two isolates increased the emergence of plants in relation to the inoculated check, but was significantly lower than the non-inoculated check. Field tests will be conducted to confirm the potential of the selected isolates to reduce the inoculum source of the pathogen.

Biocontrol activity of different *Pseudomonas* spp.
against *Verticillium dahliae* microsclerotia
Bisutti, Isabella Linda, Stephan, Dietrich 188

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Effects of elevated atmospheric carbon dioxide on the biological control
of coffee leaf rust under controlled conditions

Livia Mendes, Raquel Ghini, Wagner Bettiol 189-192

Abstract: The effect of elevated atmospheric CO₂ concentration on biological control of coffee leaf rust, caused by *Hemileia vastatrix*, was evaluated by leaf disc assay, under controlled conditions. The biocontrol agents *Bacillus subtilis*, *Bacillus pumilus* and *Lecanicillium longisporum* were applied 24h before, 24h after, and simultaneously with the *H. vastatrix* on leaf discs (diameter of 1.5cm). The CO₂ concentrations tested were: 380, 430, 700 and 1300ppm for *B. subtilis* and *B. pumilus*; and 380, 430, 670 and 1200ppm for *L. longisporum*. The antagonists were not affected by CO₂ concentrations. *B. subtilis* was the most effective in controlling the disease when applied before and simultaneously with pathogen.

Endophytic microorganisms isolated from coffee leaves, roots and branches
as plant growth promoters and biocontrol agents of coffee leaf rust

Harlen S. A. Silva, João P. L. Tozzi, César R. F. Terrasan, Wagner Bettiol 193-196

Abstract: Suppression of plant diseases and growth promotion due to the action of endophytic microorganisms has been demonstrated in several pathosystems. Experiments under controlled conditions involving 234 endophytic bacteria and fungi isolated from coffee leaves, roots and branches were conducted with the objective of evaluating the germination inhibition of *Hemileia vastatrix* urediniospores, the control of coffee leaf rust development in tests with leaf discs and on plastic bags seedling, and to promote growth of coffee seedlings. None of the fungal isolates induced plant growth or reduced disease severity. The bacterial isolates (identified by the fatty acids profile analysis) 85G (*Escherichia fergusonii*), 161G, 163G, 160G, 150G (*Acinetobacter calcoaceticus*) and 109G (*Salmonella enterica*) increased plant growth, the maximum being induced by 85G. This isolate produced *in vitro* phosphatase and indol acetic acid. In assay to control rust on coffee leaf disc, nine bacterial isolates, 64R, 137G, 3F (*Brevibacillus choshinensis*), 14F (*Salmonella enterica*), 36F (*Pectobacterium carotovorum*), 109G (*Bacillus megaterium*), 115G (*Microbacterium testaceum*), 116G and 119G (*Cedecea davisae*) significantly reduced disease severity, when applied 72 or 24h before challenging with the pathogen. In seedling tests most disease severity reduction was achieved by the isolates 109G and 119G. There was no correspondence between the organisms that promoted seedling growth and those that reduced rust severity on seedlings or leaf discs.

Biological control of avocado root rots by suppressive organic amendments
Nuria Bonilla, Blanca Landa, José María Hermoso, Jorge González,
Francisco Manuel Cazorla, Maira Martínez, Nùria Gaju, Antonio de Vicente 197-198
No abstract

Tomato proteome changes following soil application of *Pseudomonas* spp.
Pier Luigi Burzi, Anna Rita Veronesi, Roberta Roberti, Stefania Galletti,
Claudio Cerato 199-201

Abstract: Two commercial biological products Proradix® Agro (*Pseudomonas* sp.) and Salavida (*P. trivialis*) were assayed on young tomato plants under *Phytophthora infestans* challenge, under greenhouse conditions. The products were water suspended following producer's instruction and applied to the soil 6 days before the foliar inoculation with *P. infestans*. Late blight symptoms were observed 4 days after pathogen inoculation. Untreated plants appeared more infected than treated plants, but no statistically significant difference was found between treatments and controls or between the two products. However the application of each product led to a visible plant growth enhancement. A comparative study was carried out in order to highlight proteome differences among treated/untreated and inoculated/non inoculated plants. Total proteins were extracted from leaves collected 48h after pathogen inoculation and analysed by two-dimensional electrophoresis. The bioproduct application followed by the pathogen inoculation led to up/down-regulation of some protein spots which were different from those affected by the pathogen only. Mass-spectrometry analysis will be carried out in order to clarify if the observed modifications concern the activation of defence mechanisms or the observed plant growth promotion.

Characterization of site-directed mutants defective in HPR production
of *P. fluorescens* PCL1606 and its role in biocontrol
Claudia E. Calderon, Eva Arrebola, Cayo Ramos, Clara Pliego, Nuria Bonilla,
Alejandro Pérez-García, Antonio de Vicente, Francisco M. Cazorla 203-206

Abstract: *Pseudomonas fluorescens* PCL1606 is a rhizobacteria with biocontrol activity against many soil-borne phytopathogenic fungi. Its antagonist activity strongly correlates with the production of the antibiotic 2-hexyl, 5-propyl resorcinol (HPR). However, other antibiotics could be detected in *P. fluorescens* PCL1606, such as pyrrolnitrin (PRN) and hydrogen cyanide (HCN), and could be also related to its antagonist activity. To determine the role of these antibiotics on biocontrol, a collection of mutants in different antibiotics was initiated, studying in first place the genes involved in the production of HPR. A collection of site-directed mutants to the homologous *dar* operón, related to the HPR production in *P. fluorescens* PCL1606, have been constructed. Characterization of the potential traits involved in the biocontrol activity of these derivative strains were performed, including antagonism, motility, production of signal molecules, biofilm formation and biocontrol. Our results showed a role of some of the *dar* genes in the biocontrol.

STM of a biocontrol *Pseudomonas pseudoalcaligenes* strain to identify genes
involved in the interaction with *Rosellinia necatrix*
Clara Pliego, Jose I. Crespo, Cayo Ramos, Francisco M. Cazorla 207-216

Abstract: To identify bacterial genes that could contribute to biological control of the phytopathogenic fungus *Rosellinia necatrix*, 1,104 mini-Tn5-tagged insertion mutants of the biocontrol strain *Pseudomonas pseudoalcaligenes* AVO110 were screened in pools of ≤ 48 using Signature Tagged Mutagenesis (STM). One hundred and twenty nine mutants were not recovered from media containing *R. necatrix* exudates (BM-RE medium) 48 hours after inoculation, suggesting that transposon insertion in these mutants affected genes required for growth and/or survival in *R. necatrix* exudates. In a second round of STM screening, the number of mutants not recovered from BM-RE was reduced to 25 mutants. To validate the screening results, these 25 mutants were tested in BM-RE medium for competition with the wild type strain AVO110; seven

of them, were less competitive or resistant to *R. necatrix* exudates than the wild type strain and showed a single insertion of the transposon. DNA fragments flanking the Tn5 insertions in these seven mutants were cloned, sequenced and analysed using BLAST software. These genes are predicted to be involved in central metabolism, regulation and bacterial protection against environmental signals.

Biocontrol activity of *Agrobacterium* bacteriophages

Natela Toklikishvili, Tamar Eliashvili, Tinatin Khukhunashvili, George Tsertsvadze, Marina Tediashvili, Erno Szegedi, Alexander Vainshtein, Leonid Chernin 217

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Suppression of *Agrobacterium* caused crown gall disease of tomato

by rhizosphere bacteria and transgenic plants producing ACC deaminase

Natela Toklikishvili, Marina Tediashvili, Alexander Vainshtein, Susan Lurie, Erno Szegedi, Bernard R. Glick, Leonid Chernin 218

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Influence of altitude on soil microbial community variability

Paola Elisa Corneo, Alberto Pellegrini, Monika Maurhofer, Claudia Maria Oliveira Longa, Cesare Gessler, Ilaria Pertot 219-222

Abstract: The concerns on climate change and in particular the increase of global temperature are inflaming the public debate among scientist in recent years, especially regarding the possible effect on environment, animals, plants and biodiversity. Microbial populations in soil are climate-dependent. Changes in soil microbial population could influence plant growth, leading to non-visible effects in short-term on agriculture system, but dramatically changing the soil ecosystem in the long run. We aim at determining the effect of temperature and its relation with altitude on the microbial community living in three different transects of altitude cultivated with grapevines (cv. Chardonnay) in Trentino region. The final goal is to determine if temperature has a major impact on microbial richness and variability. Soil samples were collected in each altitude transect and cultivable microorganisms' abundance and total community variability was analysed. A first representative sampling has been carried out in November 2009, followed by a systematic sampling along a W-shaped design in February 2010. In February bacterial and fungal growth showed a significant difference due to the altitude ($P < 0.05$) inside each site, but not a general trend was measured comparing the three different transects. Overall comparison of bacterial and fungal growth from November to February showed a significant decrease which is attributable to the different soil temperature in different periods.

Denaturant gradient gel electrophoresis of total fungi was set up with a firstly selected set of primer to assess fungal variability. Isolation of morphologically different fungi allowed DGGE marker creation.

Biological control of *Rhizoctonia solani* and growth promotion activity of *Trichoderma koningiopsis* Th003 and *Trichoderma asperellum* Th034 formulations in potato (*Solanum tuberosum*)

Camilo Beltrán Acosta, Carlos Andrés Moreno, Paula Blanco, Laura Villamizar, Alba Marina Cotes 223-227

Abstract: Biological control agents *Trichoderma. koningiopsis* Th003 and *T. asperellum* Th034 have shown high efficacy controlling potato damping-off disease. Both isolates were formulated as water-dispersible powder (WP) and water-dispersible granule (WG) containing 1×10^{10} and 1×10^9 conidia.g⁻¹, respectively. Aqueous suspension of these biopesticides [1×10^6 conidia ml⁻¹] was applied to potato seedlings in pot trials under greenhouse conditions in order to evaluate biocontrol against *Rhizoctonia solani* and plant-growth-promoting effects. Application of Th003 WP and WG and Th034 WP resulted in 88 to 100% disease reduction. WG Th034 formulation showed the lowest efficacy (54%) of disease control. The effect of *Trichoderma* spp. on biomass accumulation, root and aerial part length, was not significant.

High population diversity of <i>Cryphonectria parasitica</i> in Croatia might hinder natural biological control of chestnut blight <i>Ježić, M., Krstić, Lj., Novak-Agbaba, S., Celepirovic, N., Rigling, D., Curković-Perica, M.</i>	228
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Selection of <i>Pichia onychis</i> isolate for biological control of <i>Botrytis cinerea</i> based on its eco-physiological characteristics <i>Carlos Andrés Moreno, Jennie Ramírez, Jimmy Zapata, Andrés Díaz, Alba Marina Cotes</i>	229-232
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Abstract: The effect of physiological parameters on growth, adherence to leaflets surface, tolerance to UV radiation and biocontrol of indigenous antagonist yeasts *Pichia onychis* (Lv027, Lv031, Lv297, Lv298, Lv299), *Pichia anomala* (Lv050) and *Candida oleophila* (Lv314) was studied. All isolates showed higher growth in culture media with acid pH (3 to 6) than the growth in basic pH conditions (≥ 8). All *P. onychis* isolates exhibited high tolerance stress conditions. Isolates Lv297 and Lv314 presented high adherence to foliar surface and isolates Lv297 and Lv050 gave effective control of *B. cinerea*. The fact that *P. onychis* Lv027 is effective biocontrol agent and tolerate a wide range of growth and stress conditions makes it an excellent candidate for production and field evaluation.

Climate effect on pathogen – biocontrol agents interaction in the tomato – powdery mildew (<i>Oidium neolycopersici</i>) pathosystem <i>Ohad Agra, Dalia Rav David, Menahem Borenshtein, Ran Shulchany, Yigal Elad</i>	233-237
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Abstract: Disease development is the cumulative effect of various factors on the host and pathogen whereby either a-biotic effects such as climate or beneficial microorganisms may affect the severity of the disease. *Oidium neolycopersici* is the causal agent of tomato powdery mildew; it causes powdery white lesions on the leaf surface, petioles and the calyx. Severe infections lead to marked reduction in fruit size and quality and to leaf wilt. A slight change in temperatures may allow either more severe disease (22 to 24°C change) or reduction in disease severity (26 to 28°C). Beneficial microorganisms can provide sufficient control, but they are sensitive to environmental conditions. A better understanding of the influence of environmental factors on pathogen – biocontrol agent interaction can help in improving their efficacy. The effect of microclimate on the development and survival of two powdery mildew antagonists was evaluated. When applied on detached leaves and incubated for 7-14 days at different temperatures, RH, and disease levels, the bacterium (B2) survival was poorer at high temperatures and low RH compared to the yeast (Y13). The microorganisms survived well at 10-15°C and high RH. Tomato plants were grown in a net house with climate regimes of high and low RH, and two disease levels were induced. Plants were sprayed weekly with the two microorganisms. The bacterium survived better under high RH as compared with lower RH and on leaves with powdery mildew as compared with symptomless leaves. The yeast was less affected by microclimate conditions, and survived well for 14 days even with a single application. We can conclude that the yeast survives better under different microclimate conditions. In the present study, various abiotic factors were found to affect different aspects of the tomato powdery mildew disease cycle. It was demonstrated that a slight change in microclimate conditions can affect the outcome of the interaction of plants with a pathogen, and of the plant-pathogen relationship with populations of control agents. The effects of climate change may be different in different plant-pathogen systems. Nevertheless, it is clear that such effects will occur and that adaptive measures need to be developed in order to respond to these expected changes.

Hyphae-colonizing bacteria – a new course of biological control agents (BCA)
against rice sheath blight disease
Cuong Nguyen Duc, Mette Haubjerg Nicolaisen, Stefan Olsson, Jan Sørensen 238
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Effect of climate parameters on induced resistance in strawberry powdery mildew
*Hellen Angelica Quiñonez Gutierrez, Yael Meller-Harel, Dalia Rav David,
Menahem Borenshtein, Ran Shulchany, Yigal Elad* 239-243

Abstract: Induced disease resistance in plants is a physiological state of enhanced defensive capacity elicited by specific stimuli, whereby the plant's innate defences are potentiated against subsequent challenges. This enhanced state of resistance is effective against a broad range of pathogens and parasites, including fungi, bacteria, viruses, and nematodes. The two most clearly defined forms of induced resistance are Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR). The changing environmental conditions may lead to changes in the activity and survival of organisms. Powdery mildew (*Podosphaera aphanis*) is one of the major fungal diseases of strawberry world-wide and is expected to increase its distribution under changing climatic conditions. In the present research we study induced resistance in strawberry plants under the changing environmental conditions that are expected due to global warming. Inducers that are tested in the present study include *Trichoderma harzianum* T39, acibenzolar-S-methyl (Bion), a new SCNB2 inducer and isolates of a bacterium and a yeast. These agents not only effectively suppressed powdery mildew on leaves when sprayed on the canopy, but also induced systemic resistance when applied to the root zone while disease was evaluated on the leaves. Similarly lower leaf application resulted in upper leaf disease suppression. Plant growth was enhanced in the presence of the biological inducers in the root zone, especially under limited irrigation conditions. The effect of different temperatures and water stress conditions on induced resistance is further studied. Furthermore, the effect of the climate parameters on the expression of induced resistance genes will be studied.

Screening of *Pseudomonas fluorescens* strains isolated from Iranian soils
for their potential to produce antifungal metabolites
and to control *Rhizoctonia* root rot of bean
Fatemeh Jamali, Abbas Sharifi-Tehrani, Monika Maurhofer 244
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Biocontrol of powdery mildew fungi by plant growth promoting rhizobacteria
*Laura García-Gutiérrez, Houda Zerrouh, Antonio de Vicente,
Alejandro Pérez-García* 245-246
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Control of pepper bacterial spot with bacteriophages
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In vitro antifungal activity of Brassicaceae spp. tissue compounds
on strawberry root pathogens
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Isolation and characterization of bacteriophages specific to *Xanthomonas euvesicatoria*
Katarina Gašić, Milan Ivanović, Anđelka Čalić, Aleksa Obradović 249
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Nicotinic acid and nicotinamide on pear and apple, analyzed in terms of cultivar and blossom age, are not limiting factors for *Erwinia amylovora* growth
Thomas Paternoster, Urska Vrhovsek, Fulvio Mattivi, Cesare Gessler, Ilaria Pertot 250
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Natural antagonists of the grapevine root rot fungus *Roesleria subterranea*
Sigrid Neuhauser, Ramona Held, Lars Huber, Martin Kirchmair 251-254

Abstract: *Roesleria subterranea* is a root-pathogenic ascomycete of grapevine causing severe economic loss in vineyards in Germany, Luxemburg and the US. Currently, no efficient control methods are available. To isolate potential fungal, antagonist soil-particles were placed onto well-established *R. subterranea* cultures. Fungi growing out of the soil particles were isolated. The majority of the isolated fungal strains belonged to the genus *Clonostachys*. These isolates together with *Trichoderma spp.* and *Clonostachys spp.* strains previously isolated from vineyard soils, endophytic isolates from diseased vines, and commercialised strains were tested in dual culture for their antagonistic effect against four *Roesleria* strains. All *Roesleria* strains reacted with an enhanced production of a green pigment in the contact zone that limited or stopped the growth of the antagonistic fungi. Most *Trichoderma* strains were able to restrict the growth of *Roesleria* and to overgrow the colony. Two out of nine *Clonostachys* strains restricted the growth of all four *Roesleria* strains. In contrast to *Trichoderma* a bleaching of the green pigment was observable when *Clonostachys* strains were growing over the *Roesleria* colonies. Whether or not rapid overgrowth of *Roesleria* colonies or bleaching of the green pigment is the key character of an effective antagonist cannot be decided at the moment. Promising strains of *Trichoderma* and *Clonostachys* will be tested in future pot experiments and field trials.

Alteration of the morphology of tomato roots due to *Fusarium oxysporum* f. sp. *lycopersici*, arbuscular mycorrhizal fungi and phosphorus supply
Alexandra Horner, Karin Hage-Ahmed, Ingrid Langer, Siegrid Steinkellner 255-258

Abstract: The wilt-inducing pathogen *Fusarium oxysporum* f.sp. *lycopersici*, a soil borne fungus occasionally causing considerable yield losses in tomato production, invades the vascular system and induces characteristic alterations in tomato roots and stems. Arbuscular mycorrhizal fungi (AMF) have been documented for their biocontrol properties; in particular they are well known to improve the plants nutritional status, mainly phosphorus. We studied the effects of *Fusarium oxysporum* f.sp. *lycopersici* and of the AMF *Glomus mossae* on tomato root morphology. Additionally the plants were treated with a nutrient solution containing different concentrations of phosphorus. Root length, root surface area (important indicators for water and nutrient uptake) and average root diameter were estimated using the image analysis program WinRHIZO. Our data indicate an alteration of these parameters depending on the treatment.

A cold-active antifungal *Pseudomonas fluorescens* isolate from Greenland
Charlotte Frydenlund Michelsen, Lisa Munk, Eigil de Neergaard, Dan Funck Jensen, Kenneth Høegh, and Peter Stougaard 259-263

Abstract: Potato soils from South Greenland contain a number of fungal-inhibiting bacteria. One isolate, *Pseudomonas fluorescens* strain In5, shows high inhibition of several plant-pathogenic fungi e.g. *Rhizoctonia solani*, *Pythium aphanidermatum*, *Fusarium graminearum* and *Phytophthora infestans* in *in vitro* inhibition assays. Also in a microcosm assay strain In5 showed the ability to suppress *R. solani*. By the use of different biochemical and genetical methods, strain In5 was found to contain the capacity to produce the antifungal compounds hydrogen cyanide (HCN) and non-ribosomal peptide (NRP). Random mutagenesis with a Tn5 derived transposon resulted in one mutant deficient in HCN production and two mutants with reduced antifungal activities.

The importance of applied biocontrol agents (BCAs) and their metabolites compared to that of the natural microbiota on strawberry
Birgit Jensen, Inge M. B. Knudsen, Dan Funck Jensen, Birgitte Andersen, Kristian Fog Nielsen, Ulf Thrane, John Larsen 264
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Screening for hydrolytic enzymes produced from indigenous bacterial isolates
Sanaa S. Kabeil, Sawsan A. Abdelatif, Sahar A. Zaki 265-273

Abstract: By screening different soil samples collected from Monofia governorate in Egypt, twelve bacterial isolates were isolated and tested against different root rot fungi namely, *Aspergillus niger* var. *niger* Tiegh., *Botrytis fabae* Sardiña, *Fusarium oxysporum* var. *orthoceras*, *Botrytis cinerea* Pers., *Fusarium avenaceum*, *Fusarium oxysporum* Schltdl., *Helminthosporium* spp., *Alternaria alternata* (Fr.) Keissl., *Fusarium solani* (Mart.) Sacc., *Bipolaris oryzae* (S. Ito & Kurib.), *Rhizoctonia solani* J. G. Kühn, *Pythium ultimum* (Trow). Different inhibition zones and different hydrolytic enzyme production obtained from these bacterial isolates. Four of them designated as D, F, E and G showed a high inhibition zone and a high production of different hydrolytic enzymes identified as genera *Pseudomonas* (D), *Bacillus* (two different isolates, E and G), and *Acinetobacter* (F); according to biochemical testes using API Kits, and confirmed with molecular identification using 16S rDNA. Isolate D (*Pseudomonas* sp.) have 96% inhibition against *Fusarium solani*. All isolates have more or less 90% inhibition against wilt diseases caused by different species of *Fusarium*. Strain E and G (*Bacillus* sp.) showed 88, 89% inhibition against these harmful fungi. Also strain F (*Acinetobacter*) showed 96% inhibition against *Pythium ultimum* causing damping off to very economic plants. High production of several hydrolytic enzymes like glucanase, protease, chitinase and lipase were detected by these bacterial isolates.

Bio-products for ecologically sound and sustainable horticultural production –
Investigations into *Enterobacter radicincitans* on radish
Beatrice Berger, Helmut Junge, Silke Ruppel 274
Abstract only

Exploiting natural biodiversity within wild olives havens for selecting rhizobacteria with antagonistic potential against *Verticillium dahliae* Kleb.
Sergio Aranda, Miguel Montes-Borrego, Rafael M. Jiménez-Díaz, Blanca B. Landa 275-278

Abstract Wild olive trees (*Olea europaea* subsp. *sylvestris*) are considered an important and unexplored source of genetic variability for traits of agronomic and biotechnological interest such as reservoirs of specific and well adapted rhizobacteria antagonistic to *Verticillium dahliae*, the causal agent of Verticillium wilt, the most important disease of cultivated olive worldwide. A polyphasic approach was used by determining the structure and diversity of bacterial communities in the rhizosphere soil and roots of 17 samples of wild olives havens in Cádiz, and Córdoba provinces in the Andalusia region at southern Spain. Results of FT-RFLP patterns of 16S rDNA sequences revealed a high heterogeneity of bacterial community composition suggesting the existence of plant genotype-site specific factors. By culture-dependent approach, 675 culturable bacterial isolates were selected and 94 of them (14%) showed different levels of antagonistic activity against the defoliating pathotype of *V. dahliae* as well as production of different enzymes and secondary metabolites. 16S rDNA gene sequences identified most of those strains as *Pseudomonas* and *Bacillus* spp.

Biochemical characterization of antifungal metabolites produced from Greek indigenous *Streptomyces*, examined as biocontrol agents
Grammatiki Kanini, Efstathios Katsifas, Dimitris G. Hatzinikolaou, Amalia D. Karagouni 279
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Identification and characterization of purple-pigmented bacteria
from rhizosphere of wild and cultivated olives in Andalucía, Spain
Sergio Aranda, Blanca B. Landa 280

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Potential climatic suitability for Fusarium wilt of chickpeas and its biocontrol agents
under current and future climatic change scenarios
Juan A. Navas-Cortés, Blanca B. Landa 281

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Biological control of soybean white mold by *Trichoderma* spp. isolates
Cleusa M. M. Lucon, Érica A. S. Pedro, Luiz A. Fukuhara 282

Abstract only

Detection of the fire blight pathogen *Erwinia amylovora* from bees
with molecular tools as part of an alternative forecasting method
Thomas Leichtfried 283-284

Abstract: Fire blight is considered the bacterial disease with the greatest economic impact in pome fruit production. The causal agent *Erwinia amylovora* can be dispersed by wind, rain, inadequate cultural measures and arthropod vectors e.g. honeybees (*Apis mellifera*). Honeybees as main pollinators in fruit crops spread the pathogen while collecting nectar and pollen from infected blossoms and could thus serve as early indicators for the presence of the bacterium in orchards. With regard to the expected global climate change, the infection conditions for *E. amylovora* could be favoured. Existing abiotic based fire blight forecasting systems would need adaptation, and monitoring of the pathogen's presence on major vectors with molecular tools could significantly improve precast of *E. amylovora* infections. As part of a master thesis, molecular tools for the detection of *E. amylovora* in honeybees were developed and tested. For a reliable detection of the pathogen in honeybees with PCR a specific purification of the bacterial DNA is necessary. An existing method for DNA extraction of microorganisms was adapted for honeybees. The sensitivity of the method could be proved by qualitative PCR in dilution series of the pathogen artificially injected in the vector. In addition, bees caught on pome fruit production sites during the growing season were investigated with the established method. To quantify *E. amylovora* in honeybees and to determine the amount of bacteria on a single honeybee a real time PCR method was used. This information about bacterial quantity is considered crucial to monitor the infection pressure on the flowering pome trees. In order to clarify the exact position of the pathogen on the vector individual parts of the bees were investigated for presence and quantity of *E. amylovora*. It could be demonstrated that the developed methods allow both, a rapid and a sensitive detection of *E. amylovora* directly from bees and that variable quantities of bacteria on different parts of the vector occur. It is proposed that this newly developed system could be applied to increase accuracy in infection prognosis of *E. amylovora* in orchards.

Lactic acid bacteria as biocontrol agents of soil-borne pathogens
Matthias Peter Lutz, Vincent Michel, Chloé Martinez, Cédric Camps 285-288

Abstract: Lactic acid bacteria (LAB) might be a novel promising bacterial group in the biological control of soil-borne pathogens. The potential of this bacterial group to suppress certain fungi has been shown in the control of post-harvest diseases of fruits and vegetables, but not much is known about their ability to protect plant roots against soil-borne pathogens. From soil and the rhizosphere of maize, rye, carrots, garden soils and compost from two origins 294 isolates of LAB have been obtained and tested in a confrontation assay against *Pythium ultimum*. In total, about 75% of the isolates showed an inhibitory effect. The most promising isolates were further tested in the pathosystem *Pythium*/tomato in pot trials. The LAB were able to protect the plants against the pathogen. Additionally the germination of tomato was significantly enhanced when the seeds were treated with LAB. Therefore we conclude that LAB are a promising bacterial group for use in the biological control of soil-borne pathogens.

Biological control of oyster mushroom green mould disease

by antagonistic *Bacillus* species

Adrienn Nagy, László Manczinger, Dóra Tombácz, Lóránt Hatvani, Júlia Gyórfi, Zsuzsanna Antal, Enikő Sajben, Csaba Vágvölgyi, László Kredics 289-293

Abstract: The occurrence of *Trichoderma pleurotum* and *T. pleuroticola* in cultivation of oyster mushroom (*Pleurotus ostreatus*) frequently results in serious crop losses. *Bacillus* strains were isolated that are very effective in antagonizing the oyster mushroom pathogenic *T. pleurotum* without having a negative effect on *P. ostreatus*. A *Bacillus subtilis* and a *B. amyloliquefaciens* strain, as well as a streptomycin-resistant derivative of a *B. licheniformis* strain proved to be very effective against the tested *T. pleurotum* strain *in vivo*. The *B. amyloliquefaciens* strain is a potential biocontrol candidate, as in addition to the lack of antagonistic activity towards *P. ostreatus*, it increased the crop yield.

Impact of genetically modified wheat on the frequency and genetic diversity of root colonizing *Pseudomonads* associated with soil fertility

Joana Meyer, Christoph Keel, Monika Maurhofer 294

Abstract only

Effectiveness of *Trichoderma* spp. in the yield of table grapes affected by *Cylindrocarpon macrodidymum* in a semi arid region of Chile

Montealegre, J. R., Sánchez, S., Pérez, L. M., Rivera, L. 295-298

Abstract: The effectiveness of two *Trichoderma* spp. formulations in the control of *Cylindrocarpon macrodidymum* affecting table grapes cropped under a semi arid climate conditions (3th Region of Chile in Copiapó), was evaluated through trials conducted during two seasons. The orchards were selected according *C. macrodidymum* damage (low growing ratio and yield of the plants). Treatments were run applying *Trichoderma* spp. into the soil as: a solid formulation, sodium alginate pellets including either a wild or an improved strain, and a liquid formulation of a commercial *Trichoderma* spp. biofungicide. An absolute control was also set. The experimental design was a complete randomized block. Results were analyzed by ANOVA and Tukey's test. *C. macrodidymum* populations, before and after treatments were compared to controls, as well as percent of yield increase and yield per plant. Survival of *Trichoderma* spp. in the soil of both formulations was also evaluated. Results showed that no statistical differences were observed in the *C. macrodidymum* populations after *Trichoderma* spp. treatments independently of the type of formulation. However, percent of yield increase was statistically significantly higher than control, when *Trichoderma* spp. formulations were applied. The *Trichoderma* populations in the soil after treatment with the solid formulations was higher than the one obtained with the liquid biofungicide. The results indicate that soil treatments with *Trichoderma* spp. in table grapes affected by *C. macrodidymum* resulted in a yield increase in treated plants cropped in a semi arid region of Chile.

Consumers' attitude to fruit produced by using biocontrol agents and climate change mitigation practices

Riccarda Moser, Ilaria Pertot, Roberta Raffaelli 299-303

Abstract: Concerns for potential risks related to the use of chemical pesticides have encouraged research of low impact alternatives as biocontrol agents (BCAs) and the implementation of a more environmentally sound agricultural management as the integrated pest management (IPM) and organic agriculture. A new regulation was recently implemented in the European Union (EC No 834/2007), which establishes the legal framework for all levels of production, distribution, control and labelling of organic products. Most of the European consumers are now familiar with IPM and organic products. Climate change is becoming a hot issue and mitigation practices are under discussion in agriculture, too. The level of consumers' awareness of mitigation practices and their willingness to pay for products produced with low carbon emission is unknown. Consumers' attitudes and preferences to fruit (apples) produced by using BCAs instead of chemical pesticides and/or climate change mitigation practices were evaluated in a choice

experiment. Preference for other apple determinants such as origin, appearance and price were also considered. The survey was administered to 96 consumers in different supermarkets in Northern Italy during fall 2009. Results show that in purchase decision, origin and price are the major determinants, followed by organic production and good appearance. Using BCAs and climate change mitigation practices increased the probability of purchasing apples. However, only coefficient associated to climate change is statistically significant and respondents are willing to pay a premium price of about 0.50 Euro/kg. Moreover, results indicate that when buying apples most people have specific requirements in mind regarding method of production, origin, appearance and price, but comparing choices made by the respondents with their individually-stated minimum requirements, the majority violated them.

Functional characterization of four *Serratia* isolates in relation to their biocontrol efficacy against *Rhizoctonia solani*
Neupane, S., Högberg, N., Alström, S., Andersson, B., Finlay, R. 304
Abstract only

Proteomic approach to characterize the biocontrol mechanism of *Trichoderma harzianum* T39 in grapevine
M. Cristina Palmieri, Michele Perazzolli, Vittoria Metafora, Angela Bachi, Ilaria Pertot 305-309
Abstract: Some biocontrol agents can activate defense mechanisms and increase grapevine resistance against pathogens. In particular *Trichoderma harzianum* T39 induces systemic resistance and significantly reduces downy mildew symptoms caused by *Plasmopara viticola*. This biocontrol agent could offer a powerful alternative to chemical pesticides, but more knowledge of the mechanisms of resistance induction is required in order to maximize its efficacy. A proteomic approach was undertaken to increase our understanding of the basis of resistance to these major pathogen and to identify potential new priming effectors in grapevine.

Mulching highbush blueberry with *Trichoderma atroviride* SC1-inoculated barks controls *Armillaria* root rot
Alberto Pellegrini, Daniele Prodorutti, Veronica Leoni, Ilaria Pertot 310
Abstract only

Effect of temperature on the antagonism between biocontrol agents and *Cylindrocarpon destructans*
Alberto Pellegrini, Daniele Prodorutti, Ilaria Pertot 311-313
Abstract: Two in vitro experiments were carried out; the first experiment compared the growth at different temperature of a strain of *Cylindrocarpon destructans* isolated in Trentino region (Northern Italy) and the second experiment tested the antagonistic activity of several biocontrol agents against *C. destructans*. *C. destructans* was plated on potato dextrose agar in the Petri dishes and incubated at different temperatures (-1, 4, 20, 23, 25 and 27°C). The growth of *C. destructans* was evaluated. In the dual-culture test we verified the activity of five different biocontrol agents against *C. destructans* at 4, 23 and 27°C. The efficacy of the biocontrol agents in inhibiting pathogen growth was measured 2, 4, 9, 14, 19, 24, 29 days after inoculation. The pathogen grew at all tested temperatures even if at different speed. *Trichoderma atroviride* SC1 was the most effective antagonist of *C. destructans* in the dual-culture test at all tested temperatures.

Survival of *Trichoderma atroviride* SC1 on grapevine pruning wounds and efficacy against Esca disease agents
Alberto Pellegrini, Veronica Leoni, Ilaria Pertot 315-318
Abstract: Esca disease is causing damage in vineyards in several parts of the world. The disease is caused by three different fungi (*Phaeoconiella chlamydospora*, *Phaeoacremonium aleophilum*

and *Fomitiporia mediterranea*), On the whole these pathogen affects the shoot of the trunk and the branches with chronic or acute evolution. At the moment the prevention of pruning wounds infection by following correct cultural practices remains the main way to manage the disease but the possibility of introducing microorganisms as biological control seems to represent an alternative or a complementary strategy. We studied the survival of *Trichoderma atroviride* SC1 on pruning wound vineyard (10^7 conidia/ml) applied during winter at two different stages (BBCH 00 and 01), and its efficacy to reduce the colonization of *Pal* and *Pch* of grapevine pruning wounds in potted plants. *T. atroviride* SC1 can survive in the wood for several months, but the percentage of colonised wounds decreases during time, probably because of the temperature rise in the vineyard. For this reason, 45 different ingredients (wetting agents, UV protectors, nutritional factors, etc.) were tested in the formulation of a semi-commercial product based on *T. atroviride* SC1 with the aim to improve its germination and persistence on pruning wounds. *T. atroviride* SC1 can successfully control wound infections of *Pch* and, at lower extent, of *Pal*.

Dissecting positive or negative effects of abiotic stress on grapevine self-protection induced by *Trichoderma harzianum* T39

Michele Perazzolli, Benedetta Roatti, Bahcine Ezzahi, Oscar Giovannini,

Ilaria Pertot 319-323

Abstract: Downy mildew is one of the most destructive grapevine diseases, and its control is based on the application of fungicides. There is increasing interest to reduce reliance on pesticides for disease control in viticulture and to focus on alternative methods, such as biocontrol agents. Several *Trichoderma* spp. strains are active against numerous plant pathogens, and their biocontrol activity is based on different mechanisms. In grapevine, *T. harzianum* T39 activates a plant-mediated resistance and reduces downy mildew symptoms, but its efficacy under abiotic stress conditions have not jet investigated. Our aim is to investigate the relationship between drought stress and *P. viticola* infection, and to characterize the efficacy of *T. harzianum* T39-induced resistance under water stress conditions.

Temperature affects antagonism of *Trichoderma* spp. against *Armillaria mellea* in soil

Claudia Maria Oliveira Longa, Ilaria Pertot 325-328

Abstract: *Armillaria mellea* root rots cause extensive economic losses on crops and in forest. Currently available chemical fungicides are ineffective in controlling the disease. Biological control, either alone or integrated with agronomic approaches may have better perspectives. Abiotic factors may affect biological control by influencing the activity of the biocontrol agents and the susceptibility of soil-borne pathogens to microbial antagonism. The effect of temperature on antagonism of *Trichoderma* spp. against *A. mellea* was evaluated in soil microcosms. Five plugs of *A. mellea* mycelium and rhizomorphs, protected by sterile lens tissue, were put between two layers of 200g of sieved sterile soil inoculated with 10^6 conidia of the testing microorganisms (*T. atroviride* SC1, *T. virens* TG1 or *T. rossicum* TG5) in polypropylene bottles (microcosms sets). Microcosm sets were maintained for seven days at 10 and 20°C. Antagonistic effect was evaluated as percentage of pathogen's growth failure on malt extract agar (reduction of vitality). *Trichoderma* spp. isolates varied in their antagonistic abilities to parasitize *A. mellea* and the antagonism was highly affected by temperature. Antagonistic ability of *T. atroviride* SC1 was low at 10°C, while the other two isolates were inactive against *A. mellea* at this temperature. Conversely at 20°C pathogen vitality was significantly reduced by the presence of each tested microorganism (Tukey's test, $P \leq 0.05$). Consortia of isolates did not increase global antagonistic ability at 10°C. Soil temperatures can differentially influence the antagonistic ability of microorganisms and this aspect is crucial in a view of climate change and increasing temperatures.

- Effect of temperature on induced systemic resistance on grape against *Plasmopara viticola* and on pathogen's population
Benedetta Roatti, Michele Perazzolli, Bahcine Ezzahi, Giovanni Brogini, Cesare Gessler, Ilaria Pertot 329-333
Abstract: Some biocontrol agents are known to induce systemic resistance (ISR) in plants reducing the severity of downy mildew disease caused by *Plasmopara viticola*, but little is known about the effect of temperature on this mechanism. Population genetic studies revealed a considerable diversity of *P. viticola* in vineyards. The aim of this study is to understand the effect of temperature on *Trichoderma harzianum* T39-induced systemic resistance on grape and to study the effect of temperature on populations of *P. viticola*. Grapevine plants treated with T39 at different temperatures are inoculated with *P. viticola* and biological effects are evaluated. Leaves are also collected for ISR/SAR genes expression analysis. *P. viticola* isolates are genotyped and phenotyped (i.e. incubation time, sporulation amount). Genotypes are co-inoculated at different temperatures and the population is analyzed with microsatellite markers and real time PCR. The results will allow a better understanding of the role of the plant and the pathogen in a view of climate change.
- The plant genotype affects the outcome of the beneficial interaction between tomato and selected *Trichoderma* spp. strains
Michelina Ruocco, Marina Tucci, Luigi De Masi, Monica de Palma, Matteo Lorito 334
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- Isolation of fluorescent *Pseudomonas* strains antagonistic to the mushroom pathogen, *Pseudomonas tolaasii*
Enikő Sajben, László Manczinger, Adrienn Nagy, Csaba Vágvölgyi 335
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- Bacterial rhamnolipids as general elicitors conferring resistance to *Botrytis cinerea* in grapevine
Sanchez, L., Varnier, A. L., Vatsa, P., Rabenoelina, F., Kauffmann, S., Pugin, A., Clément, C., Baillieul, F., Dorey, S. 336
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- Streptomyces* GB4-2 triggers rapid and widespread callose deposition in *Arabidopsis thaliana* leaves in response to fungal infection
Fengler, S., Karidaki, V., Mailaender, S., Tarkka, M. T., von Rad, U., Hampp, R., Schrey, D. 337
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- Light determines the confinement of a pathogen by an antagonistic endophyte
Schwarz, D., Fakhro, A., von Barga, S., Büttner, C., Franken, P. 338
Abstract only
- Sources of aflatoxin-degrading enzymes for potential use in decontamination of agricultural products
Natalia Zhemchuzhina, Yulia Semina, Larisa Shcherbakova, Vitaly Dzhavakhiya, Bruce Campbell 339-343
Abstract: To find microbial sources of enzymes which catabolize aflatoxin we screened 41 fungal strains, belonging to 18 genera, which co-colonize natural substrates with toxigenic *A. flavus*. Aflatoxin-catabolizing potential of each strain was assessed, *in vitro*, by cultivation for 7 days in liquid media supplemented with aflatoxin B1. After incubation, the amount of B1 remaining in the cultural filtrates was quantified by HPLC and compared to that of controls (no fungal inoculum). Among the tested strains, 28 were able to degrade B1 to varying degrees. The

most active B1 degradation was found in isolates of *Phoma glomerata*, *Cladosporium* sp., *Gliocladium roseum* and *Ulocladium* sp., which catabolized 80-98% of the B1. One time-course study on aflatoxin catabolism showed that *G. roseum* degraded almost 50% of the B1 within the first 3 days of culture. Interestingly, one biocontrol agent, *Trichoderma viride*, degraded B1 in submerged, liquid cultures only if the fungus was cultured on solid agar containing B1 prior to inoculation into liquid media.

Atoxigenic black aspergilli populations in Trentino: a natural biocontrol threaten by climate change?

Michelangelo Storari, Giovanni A. L. Broggin, Ilaria Pertot, Cesare V. Gessler 344
Abstract only

Screening for peptaibiotics in commercially available *Trichoderma* biocontrol products by LC-MS/MS

Norbert Stoppacher, Susanne Zeilinger, Rudolf Krska, Rainer Schuhmacher 345-350

Abstract: A growing number of strains of the filamentous soil fungus *Trichoderma* are commercially available as plant strengtheners, plant growth promoters or as biocontrol agents (BCA) of phytopathogens. When interacting with the pathogenic fungus and the plant, *Trichoderma* spp. produce secondary metabolites, such as peptaibiotics, which are believed to play a key role in the crosstalk between the respective organisms. Peptaibiotics are linear non-ribosomal peptides with antibiotic properties, which contain the non-proteinogenic amino acid α -aminoisobutyric acid (Aib). In this study, we screened *Trichoderma* strains present in commercial BCA products from five suppliers for their ability to produce peptaibiotics, when grown in liquid culture medium. Using an analytical workflow consisting of liquid chromatography–mass spectrometry (LC-MS) and subsequent LC–tandem MS (LC-MS/MS) experiments, we were able to detect 12 peptaibols of the group of trichotoxins, two members of the trichorzin HA group and 11 novel peptaibols in cultures of three of the investigated BCA strains.

Control of *Verticillium* wilt in pepper with two bacterial-based products and one fungal agent: a laboratory comparison

Rafael Carballeira, Javier Veloso, José Díaz 351-354

Abstract: Two commercial products, Astona™ and Botrybel™, consisting of spores of the bacteria *Bacillus pumilus* and *Bacillus velezensis*, respectively, were compared with a non-pathogenic isolate of *Fusarium* (Fo47) in the biocontrol of *Verticillium dahliae* in pepper (*Capsicum annuum*). The assays were conducted in a growth room with controlled temperature (25°C) and light (16h light – 8h dark). The biocontrol agents were applied once by dipping the roots of pepper plants in a suspension of the bacteria/fungus. 48h after this treatment, plants were inoculated with a suspension of 10⁶ conidia/ml. Symptoms as stunting, determined by stem length, were recorded weekly for 4 weeks. A significant reduction of the symptoms was observed for all the groups treated with any of the three biocontrol agents, but Fo47 was by far the most effective. The colonization of the plant by *V. dahliae* was monitored by sowing consecutive fragments of the stem in PDA supplemented with antibiotics. Samples of root and stem were collected 48h after treatment with the biocontrol agents to be further analysed. Gene expression of two defence-related genes and β -1,3-glucanase and chitinase enzyme activities were assayed searching for an evidence of the induction of plant defence by the protectant bacteria/fungus.

MBI-R20, a new pesticide, induces resistance in plants and controls both fungal and bacterial plant diseases

Hai Su, Marja Koivunen, Tim Johnson, Julie Versman, Pam Marrone 355-358

Abstract: A biofungicide formulated from *Reynoutria sachalinensis* extract, MBI-R20, controls powdery mildew and other diseases on many crops by induction of the production of plant phytoalexins and simple phenolics, and by inhibition of conidia germination. Field studies conducted in multiple locations on specialty crops such as cucurbits, tomato, lettuce, and wine grapes indicate good efficacy against major fungal and bacterial plant pathogens. In a field study

on tomato, MBI-R20 applied either alone or in a tank mix with common chemical fungicides resulted in a significant reduction in severity of bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) and reduced incidence of powdery mildew (*Oidium neolycopersici*). The efficacy was equal to that obtained with the growers' standard chemical pesticide treatments included in the study. For powdery mildew control, MBI-R20 as a stand-alone treatment (8 applications every 5-7 days) performed better at 1.0 than 0.5% concentration in this study, but both rates controlled bacterial leaf spot equally well. There were no significant differences among the MBI-R20 stand-alone, tank-mix and rotation treatments in the control of both test pathogens, which indicates that MBI-R20 can be successfully included in integrated pest management programs to decrease the risk of resistance development among plant pathogens. Preliminary results from recent field studies on wheat suggest that besides pesticidal effect against *Septoria* sp., MBI-R20 also has a beneficial effect on yield quality in terms of higher protein content and grain weight.

- P 75 Molecular and functional characterization of *Trichoderma harzianum* T39 determinants of resistance induction in grapevine
Lucie Vincenot, Michele Perazzolli, Ilaria Pertot 359-363
Abstract: *Plasmopara viticola*, the causal agent of downy mildew, is one of the most damaging diseases of grapevine (*Vitis vitifera*) worldwide. In grapevine, *Trichoderma harzianum* T39 activates a plant-mediated systemic resistance and significantly reduces downy mildew symptoms. Preliminary results suggest an implication of T39 biological status (i.e. grown conditions and ages of conidia) in the biocontrol efficacy. To further understand the mechanisms of T39-induced resistance, we aimed to characterize the fungal features for an efficient self-protection activation in grapevine. By the parallel characterization of fungal and plant genes, the impact and properties of the resistance inducer will be determined. This study will help to understand the role of physiological factors in the efficacy of biocontrol agents, in order to maximize their effect especially during changes of abiotic conditions.
- Characterization of *Trichoderma* isolates of marine origin and assessment of their potential as biocontrol agents
Inbal Gal-Hemed, Lea Atanasova, Monika Komon-Zelazowska, Irina S. Druzhinina, Christian P. Kubicek, Ada Viterbo, Oded Yarden 364
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- Development of methods to assess the efficacy of non-chemical seed treatments for control of loose smut in barley and wheat
Jan Wunderle, Eckhard Koch 365
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- Mode of action and bacterial determinants involved in the biocontrol activity of *Bacillus subtilis* towards fungal and bacterial diseases of cucurbits
Houda Zerouh, Diego Romero, Laura García-Gutiérrez, Antonio de Vicente, Alejandro Pérez-García 366
Abstract only
- Biological control of the agriculturally important weeds of southern Russia from the genera *Cynanchum* and *Vincetoxicum*
Natalia Zhemchuzhina, Marina Kiseleva, Alina Alexandrova, Elena Bilanenko, Dana Berner, Tamara Kolomiets 367-371
Abstract: Micromycetes strains were isolated from herbarium samples of weeds *Cynanchum acutum* and *Vincetoxicum funerbe* collected in the Krasnodar region a number of phytopathogenic. Strains were identified to genus and species by means of classical and molecular methods. The micromycetes *Cercospora punctiformis* Sacc. et Roum., *Alternaria alternata* (Fr.) Keissl., *Alternaria infectoria* E. G. Simmons, *Fusarium heterosporum* Nees et T. Nees, *Fusarium sporotrichioides* Sherb., *Phomopsis* sp., *Phoma glomerata* (Corda) Wollenw.

et Hochapfel, *Botrytis fascicularis* (Corda) Sacc., *Phoma pomorum* sensu auct. NZ and a *Deuterophoma* sp. were isolated from *Cynanchum acutum* tissues with disease symptoms. Fungal strains from the *Vincetoxicum funerbe* diseased tissues were identified as *Acremoniella atra* (Corda) Sacc., *Phoma eupyrena* Sacc., *Phoma chryzanthemicola* Hollos, *Fusarium oxysporum* E. F. Sm. et Swingle, *Nigrospora oryzae* (Berk. et Broome) Petch, *Curvularia geniculata* (Tracy et Earle) Boedijn, *Dendryphiella vinosa* (Berk. et M. A. Curtis) Reisinger, *Chalaropsis* (*Ceratocystis*) sp., *Alternaria* sp., *Cladosporium* sp., *Botrytis cinerea* Pers., *Gilmaniella humicola* G. L. Barron, *Verticillium dahliae* Kleb. The phytopathogenic properties of the isolated micromycetes were tested in the laboratory on the leaflets of plants. The fungi *Acremoniella atra* (Corda) Sacc. and *Phoma chryzanthemicola* Hollos. were revealed as the most perspective potential agents for the biological control of *Cynanchum acutum* and *Vincetoxicum funerbe*. Severe symptoms of damage caused by *Cercospora punctiformis* Sacc. et Roum. on *C. acutum* were noted in the Krasnodar region around Taman. It is possible this fungus also can be used as a biological control agent.

Video presentations

[Lichen-associated bacteria antagonistic to phytopathogens and their potential to accumulate polyhydroxyalkanoates](#)

[Ilona Gasser, João Vieira de Castro Junior, Henry Müller and Gabriele Berg 375-379](#)

Abstract: Bacteria are known to produce polyhydroxyalkanoates (PHA) as a storage substance, which has properties similar to those of petroleum-derived Polypropylene. Therefore, it is important to find PHA producing bacteria, which cope with industrial demands. Among terrestrial ecosystems, the highly active lichen-associated bacterial communities are expected to be a bio-resource for PHA-producers. Bacteria associated with the lichens *Peltigera canina*, *Lobaria pulmonata*, *L. immixta*, *L. virens* and *Pseudocyphellaria aurata*, collected from different European countries, were isolated and tested for their ability to antagonize the phytopathogenic fungi *Rhizoctonia solani* and *Verticillium dahliae*. Antagonists were subsequently screened on their ability to produce PHAs by applying a multiphasic approach. In a first step, a cultivation independent method was developed to determine the occurrence of gene *phaC* within the microbial community of different lichens. Using cultivation-dependent techniques, bacterial isolates from different lichens were tested on PHA accumulation *in vitro* employing a plate assay. In addition, the presence of the PHA synthase gene *phaC* was determined using PCR analysis. This study indicates that the thalli space of lichens contains a remarkable and very interesting population of PHA-producing bacteria, and more studies should be conducted to understand much better the interaction and function of these bacteria in lichens as well as their potential for biocontrol strategies. In conclusion, lichen habitats are an excellent source for PHA-accumulating bacteria.

[Volatile organic compounds of plant-associated bacteria to reduce microbial contamination on clean room textile](#)

[Martin Aichner, Lisa Oberauner, Stefan Liebminger, Michael Fürnkranz,](#)

[Gabriele Berg 381-384](#)

Abstract: Since the appearance of multi-drug resistant pathogens, it is a significant issue to find new innovative antibiotics that can solve this problem. Source for new antimicrobials are naturally occurring antagonists like plant endophytes. Bacterial plant endophytes have to protect their niche from other bacteria by producing antibiotics. Natural antagonistic endophytes from pumpkin and mistletoe were tested against the most common pathogens in clean rooms in order to identify antagonists that produce new antibiotics. The focus was on VOCs (volatile organic compounds); gaseous antibiotics emitted by bacteria, which could demonstrate a potent weapon against pathogens. Clean room textile is intended to hinder particles and microbes to contaminate the clean room environment. There is a great demand for new routes of decontamination of clean room equipment and garments. We could demonstrate for the first time that selected antagonist

showed a high activity against clean room derived pathogens and that VOC-producing antagonists were found to inhibit the growth of *Stenotrophomonas maltophilia* on clean room textiles.

Biocontrol under salinated conditions using *Stenotrophomonas* strains

Christoph Stephan Schmidt, Mohamadali Alavi, Gabriele Berg 385-390

Abstract: The genus *Stenotrophomonas* is of high medical, ecological and biotechnological interest due to the versatility of the different species. Root colonisation and plant growth promotion by *Stenotrophomonas rhizophila* DSM14405^T in different crop species and at different salinities were investigated. Plant growth promotion was most significant in solanaceous crops (tomato, sweet pepper). In non-sterile systems and ambient humidity, colonisation of above-ground plant parts was strongly dependent on plant species; epiphyllic populations on tomato were 4-5 orders of magnitude higher than epiphyllic populations on sweet pepper and cotton. Rhizosphere populations were uniformly high irrespective of plant species (10^4 CFU/g root fresh weight). However, in sweet pepper they declined sharply from 10^4 to 10^1 CFU/g root fresh weight with increasing soil salinity in non-sterile soil (0-1% NaCl). Rhizosphere populations were higher ($\sim 10^8$ CFU/g FW) and the decline with salinity less pronounced in sterile (autoclaved) soil. A similar decline with salinity could be observed in the above-ground populations. Although population sizes were lower, plant growth promotion by *S. rhizophila* DSM14405^T was more pronounced in non-sterile soil than in sterile soil. Only there the effect was consistent across all salinities so that a linear regression model with *S. rhizophila* DSM14405^T as significant growth promoting factor could be fitted. The greater effect in non-sterile soil points to an indirect plant growth promotion effect of *S. rhizophila* DSM14405^T, possibly via the elimination of deleterious components of the rhizosphere flora.

Biological control agents for combating soil-borne pathogens in Egypt

Martina Köberl, Christin Zachow, Henry Müller, Elshahat M. Ramadan, Rudolf Bauer, Gabriele Berg 391-395

Abstract: SEKEM is an Egyptian initiative and a Fair Trade company, where biologic dynamic agriculture has been practiced for more than 30 years. Medicine, which is produced from ecologically grown medicinal plants, was developed and is available worldwide. However, the cultivation of plants is more and more affected by soil-borne pathogens, which leads to significant yield losses. An environmentally friendly and sustainable solution of the problem is the introduction of biological control agents (BCA). Through the introduction of BCAs, the vitality and the natural biodiversity of the soil can be restored. To develop a specific biocontrol strategy for the SEKEM farms, microorganisms were isolated from rhizosphere and endorhiza of medicinal plants (*Matricaria chamomilla*, *Calendula officinalis* and *Solanum distichum*) as well as from Egyptian soil, and were screened for their antagonistic potential towards soil-borne phytopathogens. Promising biocontrol strains were genotypically characterized and identified.

Antagonistic endophytes from mistletoes as bio-resource to control plant as well as clean room pathogens

Gabriele Berg, Kathrin Hartenberger, Stefan Liebming, Christin Zachow 397-400

Abstract: *Viscum album* is a hemiparasitic shrub on a wide range of wood species. The bacterial and fungal communities of *Viscum album* subsp. *album* and three different host species (*Malus domestica*, *Juglans nigra* and *Acer tataricum*) were analysed by cultivation dependent methods. Endophytes were isolated from leaves and seeds of *Viscum album* as well as from branches of the host trees on five different growing media. The isolated endophytes were screened for antagonistic effects i) against plant pathogens (*Alternaria alternata*, *Botrytis cinerea*, *Phytophthora infestans*, and *Sclerotinia sclerotiorum*), and ii) against clean room inhabitants or pathogens (gram- positive bacteria: *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Paenibacillus polymyxa*, *Geobacillus stearothermophilus*, *Bacillus pumilus*; gram-negative bacteria: *Stenotrophomonas maltophilia*, *Escherichia coli*, *Pseudomonas aeruginosa*; fungi: *Verticillium dahliae*, *Aspergillus niger*, *Candida albicans*) by dual culture assay. Beside the high

proportion of antagonistic isolates against both groups in general, we found similarities but also clear differences between parasite and host. Mistletoes, especially the seeds, contained a higher antagonistic potential than the host plants. Plant-associated endophytic microorganisms from parasitic plants are an interesting bio-resource to control plant pathogens but also clean room inhabitants/pathogens.