IOBC/wprs Bulletin Vol. 78, 2012

Working Group "Biological Control of Fungal and Bacterial Plant Pathogens". Proceedings of the meeting at Graz (Austria), 07 – 10 June, 2010. Edited by: Ilaria Pertot, Yigal Elad, Cesare Gessler, A. Cini. ISBN 978-92-9067-256-2 [XXII + 400 pp.].

Climate change, biocontrol and integrated plant disease management

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.

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.

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 supression 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.

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.

New bio-resources and techniques for biocontrol

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*, *Phaeomoniella 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.

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.

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.

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

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.

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.

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.

Biocontrol agents: Ways to commercialization

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.

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.

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.

Abstract only

Short overview about new biocontrol studies

Posters

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.

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.

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.

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 complete 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 conduct to confirm the potential of the selected isolates to reduce the inoculum source of the pathogen.

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 Tn*5* 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.

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.

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 $1x10^{10}$ and $1x10^{9}$ conidia.g^{-1}, respectively. Aqueous suspension of these biopesticides [$1x10^{6}$ 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.

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.

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.

Abstract only

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. macrodydimum* 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.

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

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.*

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.

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.

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.

Abstract: To find microbial sources of enzymes which catabolaze 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.

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.

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 10^6 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.

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.

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.

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

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.

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.

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 (~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 where 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.

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.

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.