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Detoxification of the Fusarium mycotoxin zearalenone is an important trait of Clonostachys rosea in biocontrol of Fusarium foot rot of barley.

Kosawang, Chatchai; Karlsson, Magnus; Jensen, Birgit; Vélëz, Heriberto; Rasmussen, Peter Have; Collinge, David B.; Jensen, Dan Funck

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Preface	
	V
•	XIII

Abstract: The sensitivity of *Trichoderma atroviride* and *T. hamatum* biocontrol agents to a range of common soil fungi and actinomycetes was tested in co-culture on agar medium, sterile and non-sterile soil. Six test fungi (*Alternaria alternata, Aspergillus niger, Chaetomium globosum, Metarhizium anisopliae, Paecilomyces lilacinus,* and *Daldinia eschscholzii*) inhibited both *Trichoderma* species to varying degrees. In general, *T. atroviride* was more sensitive than *T. hamatum* and inhibition observed in agar culture and sterile soil was greater than that observed in non-sterile soil. Our results suggest that *Trichoderma* populations would be maintained in the soil when exposed to field levels of these test microbes and that biocontrol activity would not be compromised.

Abstract: The efficacy of the biocontrol agents (BCAs) Serenade, Prestop, Trianum P and Boniprotect Forte, applied as 4 or 5 spray programmes starting pre-flowering or at first flower for control of Botrytis cinerea fruit rot on blackcurrant cultivars Ben Hope and Ben Tirran was compared to a standard fungicide programme at similar timings and an untreated control. Programmes based on fungicides for the first 3 sprays followed by one, two or no sprays of Serenade were also included. The incidence of *B. cinerea* was assessed on green fruit samples on paraquat agar and on harvested fruit following 7 days damp incubation. The incidence of B. cinerea fruit rot pre-harvest on bushes was negligible. The incidence of B. cinerea in green fruit samples varied from 0-100% B. cinerea and in post-harvest tests varied from 0-70%. For Ben Tirran the incidence of *Botrytis* in post-harvest in untreated fruit was around 12%. All treatments, including the BCAs, significantly reduced the incidence of B. cinerea rot compared to the untreated. Best control was achieved by treatment 2 (4 x fungicide treatments) and 7 (3 early fungicides + 2 late Serenade). Replacing the fourth fungicide with two treatments with the BCA Serenade appeared to have some benefit in B. cinerea control. B. cinerea rot incidence was more variable on fruit from Ben Hope most likely due to the drier weather and hence lower botrytis risk, when this cultivar was flowering. This made the data more difficult to interpret. The lowest incidence of botrytis in the post-harvest tests was recorded in fruit from treatment 2 (4 x fungicide treatments).

Abstract: The soil-borne pathogen *Rhizoctonia solani* Kühn is responsible for serious damages on a wide range of important crops worldwide. Efficient control strategies against *R. solani* are urgently required. Application of individual biocontrol strains has often resulted in inconsistent

performance in the field. Consequently biocontrol activity was assessed by combining biocontrol strains with different modes of action to overcome this problem. The bacterial antagonist *Pseudomonas jessenii* and the mycoparasite *Trichoderma viride* were selected as promising control agents against *R. solani* the causal agent of bottom rot on lettuce. Growth chamber experiments were performed to investigate the biocontrol efficacy of individual and coinoculation treatments of these biocontrol agents in the presence and absence of *R. solani* on lettuce. Additionally, the impact of the inoculants on compositions of bacterial and fungal communities in the rhizosphere of lettuce was studied by analyzing with 16S rRNA and ITS-based fingerprinting methods. Biocontrol efficacy was improved in co-inoculants treatment of *P. jessenii* and *T. viride* compared to individual inoculant application. DGGE analysis revealed a more pronounced effect on the bacterial and fungal community in the treatment with both inoculants compared to individual bacterial and fungal inoculant application. In summary, the applied cultivation-independent methods provided insights into the complex interaction in response to the pathogen presence and to biocontrol strains inoculation.

Enc	apsulation materials and methods suitable for biocontrol of plant pathogens Anant Patel Abstract only
Dev	elopment, stability and biocontrol activity of a formulation based on <i>Pseudomonas fluorescens</i> Ps06 Jenny Carolina Ruíz, Luisa Fernanda Izquierdo, Carlos Andrés Moreno, Martha Isabel Gómez, Laura Fernanda Villamizar

Dynamics of yeasts in the phylloplane of strawberry

Abstract: Examination of the dynamics of the phylloplane yeast populations revealed immature fruits showed significantly larger populations than mature fruits or leaves. The dominant yeasts belong to the genera *Cryptococcus*, *Rhodotorula*, as well as *Sporobolomyces* and showed resistance to the two major fungicides applied to strawberry cultures. This suggests potential use of phylloplane yeasts in the integrated control of fungal diseases in strawberry.

Characterization of an alkaline serine protease of *Aureobasidium pullulans* involved in the biocontrol of postharvest diseases

Davide Spadaro, Dianpeng Zhang, Angelo Garibaldi, Maria Lodovica Gullino 33-35

Abstract: An alkaline protease gene was amplified and characterized from genomic DNA and cDNA of *Aureobasidium pullulans* PL5. Expression of ALP5 in *Escherichia coli* BL21 (DE3) yielded an homogeneous recombinant ALP5 which hydrolysed the substrate casein and inhibited the mycelial growth of the pathogens. This study provided the direct evidence that extracellular proteases secreted by the antagonist *A. pullulans* PL5 played a role in the biocontrol activities against some postharvest pathogens of apple and peach.

Abstract: Plants were inoculated with the endophytic *Penicillium* sp. and then appeared to be over sensitive to phytopathogens, which is quite original in terms of biocontrol. In this case the inoculated plants displayed stronger symptoms than the control. On the other hand an extraction of the growth medium was found to be active and provided protection to plants against

pathogens. It is likely that the benefit coming from the described endophyte will not be due to introduction of the endophyte into the plant rather using its secondary metabolites as "natural pesticides".

Effectiveness of beneficial bacteria-mediated ISR against Botrytis cinerea

in relation to priming of defense responses in grapevine

Patricia Trotel-Aziz, Bas Verhagen, Charlotte Gruau, Maryline Magnin-Robert,

Abstract: Plants have evolved the ability to enhance their basal resistance after perception of specific stimuli, such as root colonization by selective rhizobacteria or derived microbialassociated molecular patterns. In this study, we examined the differences and similarities in term of the effectiveness of various bacteria with different origins to trigger ISR and to induce or prime some defence responses in grapevine. We especially focused on oxidative burst in grapevine cell suspensions and systemic production of stilbenic phytoalexins, trans-resveratrol

First evidence of a *Lysobacter* member as a biological control agent of Plasmopara viticola

and its dehydrodimer ε-viniferin in plants.

Abstract: With the climate change, the temperature is going to play a key role in the effectiveness of microbial biocontrol agents. The influence of this environmental factor on the ecology and biocontrol activity of Bacillus amyloliquefaciens strain S499 has been investigated in this work. On this purpose, the effect of temperature on the ability to move onto solid surface, to form biofilm, to persist onto plant rhizosphere and to induce systemic resistance in plants have been evaluated in vitro and in vivo.

Biocontrol of fruit postharvest diseases by Aureobasidium pullulans

Abstract: The activity of a biological control agent, strain LL, previously identified as Aureobasidium pullulans, was tested for the first time on peach, apple and orange artificially inoculated with Monilinia laxa, M. fructicola, M. fructigena (peach) and Botrytis cinerea, Colletotrichum acutatum, Penicillium expansum (apple) and P. digitatum, P. italicum (orange). The washed cells of the antagonist were effective against all eight pathogens, inhibiting decay by over 94%; in particular the antagonist completely controlled M. laxa, M. fructicola, C. acutatum, P. digitatum and P. italicum. To our knowledge this is the first study considering the biocontrol of eight postharvest fruit pathogens with an A. pullulans strain in the same experimental conditions. Under postharvest conditions, in peaches inoculated with Monilinia spp., treated with the antagonist and stored at 0°C for 21 days plus 7 days of shelf-life, the antagonist was able to completely inhibit M. laxa and M. fructicola. In the same way, blue mould caused by P. expansum was significantly reduced on apple, after 120 days at 0°C, plus 7 days at 20°C. In in vitro trials, the VOCs released by the antagonist significantly inhibited the growth of all seven tested pathogens compared to the control, although with a different rate depending on the pathogen. These preliminary data, showing the efficacy of the A. pullulans LL strain in the control of the main postharvest fruit diseases, are promising for the development of a biofungicide for postharvest applications on a wide range of species and pathogens.

Efficacy of a biological control agent and four fungicides in the control of postharvest decay in the pear cultivar Forelle

Pieter Janse van Rensberg, Cheryl Lennox 55-57

Abstract: Main pathogens isolated from symptomatic Forelle pears were Botrytis cinerea, Penicillium expansum and Alternaria spp. Iprodione and pyrimethanil significantly reduced total decay in 2010. In 2011 fludioxonil, iprodione, pyrimethanil and pyrimethanil plus imazalil were also efficient. These fungicides decreased *Penicillium expansum*, *Alternaria* spp. effects and secondary decay caused by Botrytis cinerea. The biological control agent Cryptococcus albidus significantly reduced decay associated with a combined group of minor pathogens in 2011but was not efficient against the major pathogens in either year, indicating that postharvest fungicides tested are effective in controlling postharvest decay of pear fruit surface. However, these fungicides do not control calyx end decay caused by latent infections of *Botrytis cinerea*. In this context *Cryptococcus albidus* does not appear as an effective biological control agent for postharvest decay of Forelle pear.

Induced systemic resistance in tomato (Solanum lycopersicum)

by biochar soil amendment

Yael Meller Harel, Zeraye Haile Mehari, Yigal Elad, Dalia Rav-David,

Abstract: Grey mould, caused by *Botrytis cinerea*, is a major fungal disease of tomato (*Solanum lycopersicum*) world-wide. The aim of this study was to explore the molecular pathways involved in the induced resistance against *B. cinerea* in tomato plants grown on biochar amended medium. We observed that similar pathways of induction of defence-related gene expression take place in leaves of plants grown in biochar-amended medium independently of the biochar type and that the effect of biochar on gene expression increases with incubation time. In addition, a mutant def1, deficient in jasmonic acid lost its induced resistance ability when grown in a greenhouse biochar amended soil. This correlated with a loss of induction of expression of the genes belonging to the salicylic and jasmonic acid pathways. We conclude that Induced resistance by biochar amendment in tomato against grey mould depends on jasmonic acid synthesis.

Genomic/transcriptomic studies to optimize the biocontrol effect

of Stenotrophomonas rhizophila

Abstract: The genus *Stenotrophomonas* is of high medical, ecological and biotechnological interest due to the versatility of the different species. For example, *Stenotrophomonas rhizophila* is a model for a rhizosphere- and phylloplane- competent, salt-tolerant biocontrol agent. One of the most effective strains *S. rhizophila* DSM 14405^T showed biocontrol activity on various crops (e.g. pepper, oilseed rape, cucumber) under salinated conditions in greenhouse and field trials. Strain DSM 14405^T does not only show rhizosphere competence and antagonistic activity; it also produces high amounts of osmoprotective substances allowing it to survive under saline conditions. New insights into its mode of action are presented from transcriptomic studies based on the genome. Furthermore, this information will be used to optimise the fermentation, formulation and efficiency of the biocontrol agent.

Understanding patulin role in blue mould of apples as a tool

to improve its biological control

Simona Marianna Sanzani, Massimo Reverberi, Marta Punelli,

Abstract: Patulin ecological role has never been elucidated. Gene disruption was used to alter the sequence of 6-methyl-salicylic acid synthase. Disrupted mutants were significantly less pathogenic and virulent on apples. Mutants were more susceptible to the antioxidant quercetin than the wild type. Patulin seems to have a role in blue mould pathogenic development on apples.

Molecular strategies in biocontrol: Monitoring and optimization

of the use of Trichoderma harzianum

Josefa Blaya Fernández, Rubén López-Mondéjar, Eva Lloret Sevilla,

Abstract: The use of specific biological control agents (BCAs) has been revealed as a suitable alternative to the intensive use of fungicides in agriculture. Despite the use of these BCAs, the control of diseases in nursery plants often fails. In this work, we will present several strategies so as to improve and optimize the use of one of the most efficient antagonist, *Trichoderma harzianum*. The development of molecular methods such as qPCR and qRT-PCR, has provided

powerful tools for monitoring both active and non-active forms of *T. harzianum* in pure cultures and nursery substrates. Moreover, proper isolates of *T. harzianum* have been screened and selected according to the highest biocontrol potential and effectiveness against the target disease. To enhance the biocontrol effect of *T. harzianum*, enrichment with chitin-rich wastes has been successfully applied, decreasing the incidence of the pathogen.

Abstract: Hyphae of *Ustilago nuda* and *U. tritici* were observed to invade the shoot apical meristem and leaf primordia of barley and wheat respectively, already during the first days after the onset of germination. In a screening performed *in vitro*, a number of microorganisms and plant extracts inhibited the germination of teliospores of *U. nuda*. However, in field experiments with barley and wheat sufficient and reliable control of loose smut by seed treatment with selected agents was not obtained. On oat, seed treatment with ethanol (70%) provided a level of 75-80% control.

Influence of soil substrate on the biocontrol capacity of *Pseudomonas* CMR12a against Rhizoctonia root rot on bean (*Phaseolus vulgaris*)

Abstract: Two native isolates of rhizobacteria (*Pseudomonas migulae* Pf014 and *Bacillus amyloliquefaciens* Bs006), were selected for their effectiveness as plant growth promoters and biocontrol against *Fusarium oxysporum* in cape gooseberry in nursery, while *T. koningiopsis* Th003 only expressed biocontrol activity. However, when they were formulated and applied in field, Th003 was the most efficient biocontrol agent (5% incidence, compared to 20% in the untreated control), although rhizobacteria increased yield up to 48%.

Abstract: Fusarium Head Blight (FHB) is one of the most economically devasting disease of wheat, causing losses in yield and quality due to the presence of *Fusarium* damaged kernels and their associated mycotoxins such as the trichothecene deoxynivalenol (DON). Biological control, including treatment of crop residues with antagonists, alone or in combination in a multitrophic approach, to reduce pathogen inoculum of FHB, holds considerable promise. *Trichoderma gamsii* 6085 has been selected for its ability to grow in presence of DON and to reduce "in vitro" growth and mycotoxin production by *F. graminearum* and *F. culmorum*. *Pythium* sp. SC1-14a has been investigated for its antagonistic and competitive ability against FHB causal agents. When tested in a field trial as biocontrol agents on wheat, either as soil or spikelets inoculant, both antagonists

were able to reduce the FHB incidence. DON and other trichothecenes were at very low levels in control plots, so no information on mycotoxins reduction by the antagonists are available.

Screening of different biofungicides and optimizing the way of application
to control <i>Botrytis cinerea</i> in tomato Sarah Van Beneden, Jasper Carrette, Johan De Koker, Monica Höfte,
Geert Haesaert
Extended abstract
Study of the microbial populations on vineyards
Cátia Pinto, Remy Cardoso, Valéria Custódio, Sara Sousa, Susana Sousa
and Ana Catarina Gomes 103-107
Abstract: Vitis vinifera has a naturally complex microbial community including fungi, bacteria,
phytoplasma and viruses. A wide variety of phytopathogenic and beneficial microorganisms co-
habits with grapevine and interacts with each other. Such interactions occur both at the vineyard and during the wine fermentations and the study of the structure and the dynamics of these
microbial communities have been neglected over the last years. Moreover, it is important to note
that the presence of specific microbial community will influence the wine production and quality.
In this study, we investigated the composition of the natural grapevine microbiome from <i>Tinta</i>
Roriz variety during the vegetative cycle, using a biodiversity based metagenomics' approach.
A high diversity of Eukaryotic and Prokaryotic population was uncovered. Interestingly,
grapevine displays a major occurrence of the eukaryotic Aureobasidium and the prokaryotic
Enterobacteriaceae. Our results expose a high and dynamic biodiversity during the grapevine
vegetative cycle.
Transcriptional reprogramming of the mycoparasitic fungus Ampelomyces quisqualis
during host recognition
Stefanos Siozios, Lorenzo Tosi, Alberto Ferrarini, Alessandro Ferrari,
Paola Tononi, Diana Bellin, Monika Maurhofer, Cesare Gessler, Massimo Delledonne, Ilaria Pertot
Extended abstract
Plant signalling pathways and bacterial determinants involved in the induction
of systemic resistance triggered by <i>Bacillus subtilis</i> UMAF6639
Laura García-Gutiérrez, Houda Zeriou, Antonio de Vicente,
Alejandro Pérez-García 111-112
Extended abstract
Isotopic-labelling assisted metabolomics of the biocontrol fungus <i>Trichoderma</i>
Rainer Schuhmacher, Maria Doppler, Bernhard Kluger, Susanne Zeilinger,
Rudolf Krska 113-114
Extended abstract
Antifungal 2-hexyl, 5-propyl resorcinol is responsible for the biocontrol ability
of Pseudomonas fluorescens PCL1606
Claudia E. Calderón, Eva Arrebola, Jose A. Gutiérrez-Barranquero,
J. Ignacio Crespo-Gómez, Antonio de Vicente, Francisco M. Cazorla
Abstract: The biocontrol rhizobacterium <i>Pseudomonas fluorescens</i> PCL1606 has strong
antagonistic activity against many soil-borne phytopathogenic fungi, including <i>Rosellinia</i> necatrix, the causal agent of white root rots of many plants and Fusarium oxysporum f. sp.
radicis-lycopersici, which causes the tomato foot and root rot The relevant characteristic of this
bacterium is the production of the antifungal antibiotic 2-hexyl, 5-propyl resorcinol (HPR).
Analysis of this genomic clone resulted in the presence of five homologous to dar genes with an
organization that resembles to that previously described on <i>P. aurantiaca</i> BL915. The role of

those dar-homologous genes in HPR production, and in the biocontrol activity is described in this work.

Biostimulation of the bacteria degrading quorum-sensing (QS) signals for quenching the QS-dependent expression of the virulence symptoms caused by <i>Pectobacterium</i> on potato plants Anthony Kwasiborski, Nicolas Mothe, Yannick Raoul des Essarts,	
Amélie Beury-Cirou, Denis Faure	25
Extended abstract	
Effect of a fungal infection on the volatile compounds emitted by barley's roots and their roles in the interactions in the rhizosphere Marie Fiers, Fanny Barsics, Georges Lognay, Jean-Paul Wathelet, Marie-Laure Fauconnier, M. Haïssam Jijakli	28
Extended abstract	
Dissecting the tripartite interaction between <i>Vitis vinifera</i> , <i>Plasmopara viticola</i> and the biocontrol agent <i>Trichoderma harzianum</i> T39 M. Cristina Palmieri, Michele Perazzolli, Vittoria Matafora, Angela Bachi,	
Ilaria Pertot	31
No abstract	
Detayification of the Eugarium myostovin zoorolonoma is an important trait	
Detoxification of the <i>Fusarium</i> mycotoxin zearalenone is an important trait of <i>Clonostachys rosea</i> in biocontrol of Fusarium foot rot of barley	
Chatchai Kosawang, Magnus Karlsson, Birgit Jensen, Heriberto Vélëz,	
Peter Have Rasmussen, David B. Collinge and Dan Funck Jensen 133-13	
Abstract: The fungus Clonostachys rosea 'IK726' has proven to be effective in biologic	
control of a range of plant diseases. Among these are fusariose caused by Fusarium graminearu	
or Fusarium culmorum – pathogens known to produce mycotoxins such as zearalenone (ZEN and deoxynivalenol (DON). It has been shown that the zhd101 gene in C. rosea encode	
zearalenone lactonohydrolase (ZHD) which breaks down the <i>Fusarium</i> mycotoxin (ZEN) to le	
toxic derivates in vitro. However, it has not been determined previously whether ZE	
detoxification plays a role in biocontrol of F . graminearum on plants. Here we show th	
C. rosea, via detoxification, counteracts exposure to ZEN during biological control interaction	
with F. graminearum on barley seedlings and that this is an important trait for the efficacy	
biological control performance.	
The Trichoderma atroviride Eng18B ENGase gene product is essential	
for <i>in vitro</i> antagonism against <i>Botrytis cinerea</i>	

Mukesh K. Dubey, Wimal Ubhayasekera, Mats Sandgren,

recovered by re-introduction of an intact *Eng18B* gene fragment in mutant strains. A putative role of Eng18B ENGase activity in the endoplasmic reticulum associated protein degradation pathway of endogenous glycoproteins in *T. atroviride* is discussed in relation to the observed phenotypes.

Yaseen Thaer, Anna Maria D'Onghia, Alessandra Ricelli
Importance of surfactin for plant resistance induction by Bacillus isolates Hélène Cawoy, Martin Mariutto, Emmanuel Jourdan, Philippe Thonart, Marc Ongena
Screening ELISA method to assess the <i>in vitro</i> efficacy of essential oil against damageable European phytopathogens Olivier Parisi, Abdesselam Zhiri, Dominique Baudoux, Haissam Jijakli
A combined oligochitosan and oligopectin elicitor triggers plant defense and confers protection against a wide range of plant pathogens Géraldine van Aubel, Raffaele Buonatesta, Pierre Van Cutsem
The lactoperoxydase system, a natural and biological control of potato late blight Françoise Bafort, Jean-Paul Perraudin, Nicolas Flament, M. Haïssam Jijakli
Relationship between the aggressiveness of <i>Botrytis cinerea</i> on tomato and the efficacy of biocontrol <i>Marc Bardin, Morgane Comby, Claire Troulet, Philippe C. Nicot</i>

median = 49%). A correlation was observed between the level of aggressiveness of a strain to tomato and its sensitivity to the biocontrol agent (assessed by the protection level). It reveals the importance of considering several strains of the pathogen when screening for biocontrol agents, to obtain a good representation of the pathogen population and thus take into account the

potential durability of biocontrol.

The use of ozone in strawberry post harvest conservation

Endophytic ability of <i>Trichoderma</i> spp. as inoculants for ornamental plants innovative substrates <i>Domenico Prisa, Sabrina Sarrocco, Maurizio Forti, Gianluca Burchi, Giovanni Vannacci</i>
Optimisation of time of application of <i>Trichoderma</i> bio-control agents for grapevine pruning wound protection Cheusi Mutawila, Francois Halleen, Lizel Mostert
Induction of systemic resistance against <i>Cucumber mosaic virus</i> and bacterial speck pathogen by <i>Penicillium simplicissimum</i> GP17-2 in <i>Arabidopsis</i> and tobacco <i>Mohsen Mohamed Elsharkawy, Md. Motaher Hossain, Masafumi Shimizu, Mitsuro Hyakumachi</i>
Screening of bacteria and fungi antagonist to <i>Phytophthora</i> and <i>Pythium</i> species pathogenic of forest trees François Lefort, Thibaut Pralon, Justyna Nowakowska, Tomasz Oszako
Population of antagonistic actinomycetes and bacteria against Fusarium oxysporum in the rhizosphere of cucumber and Welsh onion Yoko Suzuki, Masafumi Shimizu, Mitsuro Hyakumachi
Trichoderma harzianum 6776, a promising biocontrol agent and plant growth promoter Sabrina Sarrocco, Lorenzo Moncini, Giampaola Pachetti, Giovanni Vannacci

Increasing efficacy of <i>Ampelomyces quisqualis</i> against powdery mildew pathogen	
Dario Angeli, Krishna Saharan, Monika Maurhofer, Cesare Gessler,	
llaria Pertot	195-196
Extended abstract	

Efficacy of some *Bacillus* antagonist for controlling *Alternaria brassicicola*, causal agent of Chinese kale leaf spot

Boossaracum Udomsak, Sureeporn Bua-art, Nuttima Kositcharoenkul,

and field trials. In the laboratory, one hundred and thirty five isolates of *Bacillus* sp. isolated from soil, manual and planting material, were tested on a A. brassicicola fungus for growth inhibition character by dual plate technique. The results revealed that 90 isolates could inhibit the A. brassicicola mycerial growth on PDA medium and 5 isolates showed high effectiveness. In screen house test, spraying with cell suspension of the 5 Bacillus sp. isolates showed that all of them could reduce leaf spot symptom on Chinese kale when compared to water spraying treatment. Among them, isolate 20W12 showed the highest effectiveness in controlling Chinese kale leaf spot. Under field condition, cell suspension spraying test were conducted during dry period (December, 2010 to February, 2011) and rainy period (June to August, 2011) at Kanchanaburi province. Eight treatments with 4 replications in RCBD; spraying with each of 5 cell suspension of Bacillus sp. isolates, A. brassicicola inoculation (C+), water spraying (C-) and spraying with solution of 40 grams of mancozeb 80% WP in 20 liters of water, on 35 days old Chinese kale plants, 48 hours before and 48 hours after A. brassicicola inoculation were used. During January to March, 2012, the 5 Bacillus sp. isolates in the form of wettable powder suspension were conducted at the same field as previous trials. The results showed that in dry period, 5 isolates of Bacillus sp. cell suspension could reduce the disease better than water spraying treatment, but they have no statistical difference when compared to mancozeb treatment. These 3 isolates which showed the highest efficacy in dry period were 17G18 20W5 20W1 and in rainy period were 20W4 20W1 and 20W12. The results from applying with wettable powder formulation of isolates 20W1 20W5 and 17G18 showed highly effective. Identification of those 5 Bacillus isolates by API technique revealed that 20W5 20W12 20W4 and 20W1 were B. subtilis and 17G18 was B. licheniformis.

Brazilian diversity of *Trichoderma* spp. and isolate selection

Extended abstract

Trichoderma harzianum seed treatment combined with biofumigation

by *Brassica carinata* meal to reduce lettuce damping off by *Pythium ultimum Stefania Galletti, Pier Luigi Burzi, Stefano Cianchetta, Claudio Cerato* 203-208

Abstract: Seed treatment by biological control agents, like the filamentous fungus *Trichoderma* spp. represents a valuable option to control those pathogens which attack seedlings, causing preand post-emergence damping off, like *Pythium* spp. Another sustainable tool for the control of soilborne pathogens is represented by the biofumigation technique which is based on the soil amendment with Brassicaceae derived products, through the green manuring of cover crops or seed meal amending. The aim of this work was to evaluate the effect of combining the seed treatment by *Trichoderma harzianum* isolate with the biofumigation by *Brassica carinata* meal, to control damping off of lettuce. The experiments were carried out in two types of soil, artificially inoculated by a pathogenic isolate of *P. ultimum*. The *T. harzianum* isolate was previously selected for tolerance to biofumigation compounds and it was applied to seeds as

conidia suspension or liquid culture. In the first type of soil only liquid culture seed treatment significantly increased the percentage of healthy plantlets, irrespective to biofumigation, suggesting a role for the metabolites produced during *T. harzianum in vitro* growth. These results were substantially confirmed also with the second type of soil.

Trichoderma harzianum T39 biocontrol activity against downy mildew: cytology of the direct and plant-mediated mechanisms M. Cristina Palmieri, Michele Perazzolli, Ilaria Pertot	209-210
Extended abstract	
Role of <i>Verticillium tricorpus</i> in the interaction of <i>Verticillium longisporum</i> with cauliflower	
Lien Tyvaert, Soraya C. França, Jane Debode, Monica Höfte Extended abstract	211-212
Biocontrol of <i>Alternaria brassicicola</i> on cabbage seedlings and	

Abstract: Endophytic Streptomyces sp. strain MBCN152-1 which was isolated from cabbage greatly reduced disease severity on plug-seedlings of cabbage after spray-inoculation with Alternaria brassicicola. Furthermore, amendment of growing medium with MBCN152-1 strongly inhibited the incidence of damping-off of cabbage seedlings raised from seeds artificially infested with A. brassicicola. Microscopic observation revealed that hyperparasitism is one of the modes of action of strain MBCN152-1. These results indicated that strain MBCN152-1 is a suitable candidate for the biological control of A. brassicicola. Endophytic actinomycetes, isolated from strawberry plants, were tested for inhibition of anthracnose, caused by Glomerella cingulata, on detached leaflets and plug seedlings of strawberry. Streptomyces sp. strain MBFA-172 strongly inhibited symptom development on the detached leaflets and significantly reduced disease incidence on strawberry plug seedlings. Strain MBFA-172 provided higher level of protection than a commercial biofungicide (Talaromyces flavus) and comparable level with a chemical fungicide (propineb) under greenhouse conditions. A spontaneous thiostrepton-resistant mutant of MBFA-172 colonized aboveground parts of strawberry plants at mean population densities of 3.2×10^3 to 7.2×10^5 cfu/g (fresh weight) even after 21 days of foliar application. These results indicated that MBFA-172 has a great potential as a biocontrol agent for strawberry anthracnose.

The effect of the biological control agent *Trichoderma harzianum* T-22 on spot blotch disease in Libyan barley cultivars in a field experiment *Abdallah M. Aada, Ethan Hack* 219-225

Abstract: Spot blotch caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is a serious disease of barley in Libya. A small-scale field experiment was carried out in the UK to test the effectiveness of *Trichoderma harzianum* T-22 for controlling spot blotch in two Libyan barley cultivars, Nibola and Rehan. Nibola was significantly more resistant to spot blotch than Rehan. Two methods of applying T-22, foliar spray and seed coating, were tested individually and in combination. There was some reduction in disease severity with all three application methods, but the combined treatment most consistently reduced disease severity and increased yields of both cultivars. The results provide evidence that combining disease resistance with biological control may be beneficial for spot blotch management.

Development of a TaqMan® probe-based real-time PCR approach to track the biological control agent *Pseudomonas poae* RE*1-1-14 Gabriele Berg, Heidemarie Pirker, Henry Müller, Ralf Tilcher,

Abstract: The sugar beet endophyte Pseudomonas poae RE*1-1-14 is a component of an antagonistic cocktail developed to control root rot diseases in sugar beet. Based on an extended screening process the microbial compilation also includes the rhizobacteria P. fluorescens L13-6-12 and Serratia plymuthica 3Re4-18. To assess cell numbers during the seed treatment procedure, subsequent storage of the inoculated seeds and, finally, in course of plant development, a quantitative real-time PCR method was established for the model strain P. poae RE*1-1-14. For cultivation-independent monitoring of the population dynamics of the strain, we developed a pair of SCAR (Sequence Characterized Amplified Regions) primer for a specific 580 bp fragment derived from a comparative U-PCR approach. In combination with a strainspecific probe, the primer set was suitable to be employed in a TaqMan® real-time PCR assay. The described system allows the accurate detection and quantification of the antagonist in sugar beet seed coatings and ad planta under greenhouse conditions.

Enhancement of biological control properties naturally present in soil

Extended abstract

Effect of plant essential oils and biocontrol agents on infection of groundnut by Aspergillus flavus

Abstract: Aspergillus flavus is a major pathogen of groundnut in many countries, including Nigeria. Infection can result in contamination of groundnut seed with the mycotoxin, aflatoxin, which poses a potent threat to the health of individually consuming infected nuts. In preliminary experiments twelve plant oils were screened for inhibition of A. flavus on amended PDA plates. Only four extacts; clove, camphor, garlic, and galangal oils showed high inhibitory effects. Clove oil and camphor oil also significantly suppressed infection of groundnuts by A. flavus in amended compost, when applied as seed treatments. Biological control based on use of *Trichoderma* spp. (T. harzianum, T. viride and T. asperellum), Bacillus amyloliquifaciens (isolates MBI 600, 62P and 66P) and Pseudomonas chlorophis was also evaluated. In vitro assays on agar plates showed the Trichoderma spp. and Bacillus isolates 62P and 66P were the most active. These same organisms were also effective in improving emergence when applied as a seed amendment to seed sown in Aspergillus-inoculated compost. When applied as a preventative treatment to groundnut pods, the *Trichoderma* spp. were also effective in suppressing infection, when the pods were subsequently inoculated with A. flavus. A strategy may be therefore be possible which provides control of A. flavus on groundnut, using BCAs and plant extracts, applied alone or in combination.

Development of suppressiveness to root rot caused by Cylindrocladium spathiphylli in container media amended with marine residues

Abstract: This study aimed at evaluating the potential of fish hydrolyzed (FH), fish emulsion (FE), shrimp peel powder (SP), Sargassum seaweed powder (SW) and mussel shell powder (MS) to induce suppressiveness against Cylindrocladium spathiphylli in container media. In the container media, naturally infested, FH and FE at concentrations of 0, 5, 10, 15, 20 and 25% (v/v) of the volume of water required to reach the water retention capacity of the container media, and SP, SW and MS at concentrations of 0, 1, 2, 3, 4 and 5% (v/v) were incorporated. The mixtures were incubated for 10 days at room temperature. Afterwards, the mixtures were transferred to plastic pots of 300 ml, followed by the planting of one plug of Spathiphyllum 'Opal'/pot. When planting, at 90 and 180 days of cultivation, microbial respiration, carbon microbial biomass (MBC), and fluorescein diacetate hydrolysis (FDA) were determined. The incidence was

evaluated after 180 days and disease severity was assessed weekly throughout until 180 days. To confirm the causal agent of disease in plants, root fragments were placed in Petri dishes containing PDA + streptomycin, kept in a growth chamber. Fish hydrolyzed, FE, and SP reduced the incidence and the area under curve of progress disease (AUCPD). Fish emulsion at 20% showed the highest dry matter and the lowers recurrence of the pathogen in 180 days. Hydrolysis of diacetate of fluorescein, respiration of container media, and carbon microbial biomass showed quadratic effect at concentrations of FH, FE and SP, with the inflection point at 25, 20 and 3%, respectively. We concluded that FE at 20% (v/v) promoted the greatest suppressiveness to *C. spathiphylli*, as well as increased plant development.

Effects of phenazines on <i>Pseudomonas</i> sp. CMR12a cell physiology under microaerobic conditions	
Chien-Jui Huang , Katrien De Maeyer, and Monica Höfte	'-248
Chitinase isoforms in zucchini leaves treated by <i>Nostoc</i> sp. extract <i>Roberta Roberti, Pier Luigi Burzi, Hillary Righini, Carolina Perez Reyes, Stefania Galletti, Guillermo Garcia-Blairsy Reina, Agostino Brunelli</i>	plant of the gainst earch foliar it is a costoc were t, and three
Elicitation of grapevine defense responses against <i>Plasmopara viticola</i> , the causal agent of downy mildew Moustafa Selim, Gregor Langen, Beate Berkelmann-Löhnertz, Karl-Heinz Kogel, Danièle Evers	-254
Jtilization of solid and liquid <i>Trichoderma harzianum</i> formulations to biocontrol <i>Pyrenochaeta lycopersici Soledad Sánchez, Rodrigo Herrera, Ximena Besoain, Luz María Pérez, Jaime R. Montealegre</i>	-256
Preliminary tests in field conditions of alternatives substances against grape downy mildew in organic farming Ingrid Arnault, Marc Chovelon, Sylvie Derridj	

Abstract: Within the context of research of alternatives to the use of copper against the grapevine downy mildew (*Plasmopara viticola*), screening tests have been carried out to assess the activity of potential products either as fungicidal or as elicitors of plant defense and/or resistance. Three hydroalcoholic solutions of plant extracts *Salix alba*, *Equisetum arvense*, *Artemisia vulgaris* and three aqueous ones of *Frangula alnus*, *Rheum palmatum* and of microdoses of D-fructose (100ppm) were sprayed single on leaves or associated with 100g/ha of copper hydroxide. Their efficacies were evaluated versus 600g/ha of copper hydroxide and an untreated control. The trials were randomized and carried under shading nets on two vine

cultivars planted in individual pots: Vitis vinifera cv. Alphonse Lavallée grafted onto 110 Richter and V. vinifera cv Muscat de Hambourd grafted onto 161-149 Couderc. In 2011 the product efficacies expressed by the attack frequencies and intensities of P. viticola, showed that the solutions of F. alnus, E. arvense and of D-fructose associated with copper 100g/ha, were as effective as copper hydroxide 600g/ha. They significantly reduced attack rates versus the untreated control. A. vulgaris extract had no visible effects. Further experiments will be conducted in 2012 to corroborate these interesting data.

Development of a biopesticide prototype based on the yeast

Rhodotorula glutinis Lv316 for controlling Botrytis cinerea in blackberry Jimmy Zapata, Laura Villamizar, Andrés Díaz, Liz Uribe, Carol Bolaños,

Abstract: A biopesticide prototype based on a strain of *Rhodotorula glutinis* Lv316 that exhibit a high biocontrol activity against Botrytis cinerea was formulated as a concentrate suspension. A culture medium was developed to reach a yield of 6×10^9 cell/ml in batch fermenter for both 13-1. Two biopesticide prototypes were developed, one of them was supplemented with an optical brighteners which conferred a high UV-B protection (62%). Both presented high biocontrol activity since their efficacy ranged from 55 to 65% in a blackberry commercial crop, compared with 45 and 26% disease reduction using a chemical treatment diffenoconazol and carbendazim, respectively.

Biocontrol and biofertilizer activities of the Streptomyces anulatus S37:

an endophytic actinomycete with biocontrol and plant-growth promoting activities Olivier Couillerot, Parul Vatsa, Souad Logman, Yedir Ouhdouch, Hubert Jane, Jean-Hugues Renault, Christophe Clément and Essaïd Ait Barka 271-276

Abstract: Streptomyces anulatus S37 is an actinomycete able to colonize grapevine in-vitro plants and inhibit the growth of Botrytis cinerea. This strain has also a great potential to produce antifungal metabolites. These results indicate the potential of developing effective actinomycetes from Moroccan habitats for the biological control of B. cinerea. Further studies are ongoing to better understand the activity of each of the purified molecules.

Performance of biocontrol products in protecting tomato plants

against Botrytis cinerea under controlled conditions

Abstract only

Effects of UV-B radiation on the antagonistic ability of *Clonostachys rosea*

to Botrytis cinerea on strawberry leaf disc

Wagner Bettiol, Lúcio B. Costa, Marcelo A. B. Morandi,

Abstract: Human activities are altering the concentrations of ozone in the troposphere and hence in the incidence of ultraviolet-B (UV-B) on Earth's surface. Although representing only five percent of UV-B radiation striking the Earth's surface, this radiation has the potential to cause effects on biologically active molecules. Sensitivity to UV-B radiation is one of the limitations of biological control of plant pathogens in the field. The objectives of this work were to evaluate the effects of UV-B on several isolates of Clonostachys rosea, and the ability of an isolate of C. rosea, previously selected for its tolerant to UV-B radiation, to control Botrytis cinerea on strawberry leaves in controlled conditions (strawberry leaf discs). The germination of C. rosea conidia was inversely proportional to the irradiance. The most tolerant strain (LOC62) had relative germination of about 60% after irradiation of 4.2kJ/m², and this strain was selected to be used in the subsequent studies. The data showed that even with exposure to UV-B radiation, C. rosea LQC62 controlled the pathogen. Conidial concentrations of strain LQC62 above 10⁵ conidia/ml showed higher tolerance to UV-B radiation and increased ability to control more than 75% of the B. cinerea even with exposure to radiation. According to our results, in addition to

showing less growth under UV-B, conidia of *C. rosea* had lower antagonistic ability. Further studies are needed to observe the tolerance of *B. cinerea* conidia to UV-B radiation and thereby prove that an environment with increased UV-B radiation may be favoring the pathogen due to a lower ability of *C. rosea* to control the pathogen in conditions of increased UV-B.

Psychrophilic *Trichoderma* isolates from Subpolar Russia:

in vitro antagonism against Rhizoctonia solani

Tatiana Suprunova, Natalya Shmykova, Daria Shumilina,

Larisa Sherbakova, Tatyana Smetanina, Boris Borisov, Alina Aleksandrova,

Abstract: *Trichoderma* isolates were isolated from soil samples collected at the Nenets Autonomous Area, a region of Russia with extremely cold climatic conditions. Nine *Trichoderma* isolates were evaluated for their antagonistic properties against *Rhizoctonia solani* using an *in vitro* challenge plate assay and incubation temperatures ranging from 12 to 22°C. Four isolates with psychrophilic properties were selected for further study as potential biological control agents. Study of the mechanisms of antagonism by these isolates is ongoing.

Insights on the role played by temperature on the biocontrol agent

Bacillus amyloliquefaciens strain S499

Abstract: With the climate change, the temperature is going to play a key role in the effectiveness of microbial biocontrol agents. The influence of this environmental factor on the ecology and biocontrol activity of *Bacillus amyloliquefaciens* strain S499 has been investigated in this work. On this purpose, the effect of temperature on the ability to move onto solid surface, to form biofilm, to persist onto plant rhizosphere and to induce systemic resistance in plants have been evaluated *in vitro* and *in vivo*.

Influence of combined environmental parameters on survival

of the biocontrol agent *Penicillium oxalicum* 212 in different soil types *Inmaculada Larena, Gema Vázquez, Antonieta De Cal, Paloma Melgarejo,*

Abstract: In order to optimize the practical application of *Penicillium oxalicum* strain 212 as a biological soil treatment, and to achieve an optimal biocontrol efficacy, it is essential to understand how the physical environment (temperature, water availability and soil texture) influences the biocontrol agents' growth and survival. The combined method, i.e., the counting of colony forming units (CFU) on semi-selective medium and quantitative real-time PCR (qPCR), used in this work are useful for tracking PO212 in different soil types. PO212 grew in all studied soils, temperatures and water potentials, although its growth was better in soil with low water availability than in those soils with medium and high water availability.

Evaluation of treatments to control ripe rot caused by

Colletotrichum acutatum Simmonds in blueberry

Extended abstract

Enhancement of biocontrol efficacy against *Botrytis cinerea*

through the manipulation of nitrogen fertilization of tomato plants

Manzoor Ali Abro, François Lecompte, Marc Bardin,

Abstract: Although nitrogen fertilization is known to affect plant susceptibility to certain pathogens, little is known on its possible effect on the efficacy of biological control. In the present study we examined the effect of five levels of NO_3 nutrition on the efficacy of two biocontrol agents (*Trichoderma harzianum* and *Microdochium dimerum*) to protect pruning wounds of tomato against *Botrytis cinerea*. Plants were grown for two months in a greenhouse

with a soil-less drip-irrigation system. Differential nitrogen nutrition was applied for the last four weeks prior to pruning, treatment of wounds with the biocontrol agents and inoculation with two strains of *B. cinerea*. They were then incubated in conditions conducive to disease development. Plant fertilization had a highly significant effect on disease development for both strains tested and it significantly influenced the efficacy of both biocontrol agents. High nitrogen fertilization generally decreased disease severity and also enhanced the efficacy biocontrol.

Prediction of transcriptional regulatory elements for systemic resistance

induced by a plant growth promoting fungus

Penicillium simplicissimum GP17-2 based on microarray data

Yohei Yoshioka, Naznin Most. Hushna Ara, Ayaka Hieno, Masafumi Shimizu,

Abstract: The plant growth promoting fungus (PGPF) *Penicillium simplicissimum* GP17-2 induces systemic resistance against fungal and bacterial pathogens. The induced systemic resistance (ISR) signalling involves multiple plant hormone mediated paths, including salicylic acid, jasmonic acid, and ethylene. In this study, we investigated the signal transduction of GP17-2-mediated ISR by microarray and comprehensive *cis*-regulatory elements prediction. The prediction provided the candidates of various putative ISR and also plant hormone-responsive elements. Signalling cross-talk among GP17-2 and plant hormone responses including salicylic acid, hydrogen peroxide and abscisic acid were detected by comparative microarray analysis and *cis*-regulatory elements prediction. These results provide that new knowledge of transcriptional network of GP17-2-mediated ISR and plant hormone signalling.

A rapid test to detect exo-chitinase activity for soil microorganisms using

4-methylumbelliferyl-N-acetyl-β-D-glucosaminide

Extended abstract

Development of mass production technology for a new strain of

Ampelomyces quisqualis for use as mycopesticide

Abstract: The mycoparasite Ampelomyces quisqualis strain ITA3 is a biocontrol agent of powdery mildew disease but the mycelium and conidia production of the fungus still needs to be developed. The objective of this research is to find out the optimum conditions for high mycelium and conidia production of the strain ITA3. At submerged-fermentation stage (vegetative growth), the nutrition factors (carbon source, nitrogen source and C/N ratio) were optimized whereas at solid surface-fermentation stage (sporulation) culture nutrient medium was optimized for lower cost. The results showed that, out of three kinds of carbon source, sucrose (jaggery) gave the highest mycelia growth compared to dextrose and glucose. The organic nitrogen sources (i.e. potato extract, yeast extract, malt extract, urea) gave a better significant growth for A. quisqualis strain ITA3 (P < 0.001) compared with the inorganic nitrogen source (sodium nitrate, ammonium chloride). The C/N ratio and agitation speed were also optimized for higher mycelium growth of the fungus. It was observed that the harvested mycelium from stationary growth phase of ITA3 (6 days) transferred into a solid potato extract agar plate gave higher sporulation (4 days) in comparison to transferring it into solid jaggery and potato dextrose agar plates. At this optimum condition the strain ITA3 produced an average of 5 x 10⁸ conidia per g of mycelium after 96 hours. These outcomes indicate that the higher mycelium and spore production of A. quisqualis strain ITA3 can be achieved with a two-step fermentation system by the short period with cheaper culture medium.

Targeted screening of iron-containing metabolites (siderophores) from <i>Trichoderma</i> spp., <i>A. alternata</i> and <i>A. niger</i> using LC-HR-MS/MS on an LTQ Orbitrap XL
S. M. Lehner, N. K. N. Neumann, L. Atanasova, R. Krska, M. Lemmens, I. S. Druzhinina, R. Schuhmacher
Extended abstract
EU-project CO-FREE: Innovative strategies for copper-free low input and organic farming systems Annegret Schmitt, Ilaria Pertot, Jürgen Köhl, Aimilia Markellou, Didier Andrivon, Jolanta Kowalska, Krotoum Konate, Markus Kelderer, Edith Lammerts van Bueren, Christian Bruns, Jo Smith, Annabel Simon-Levert, Philippe Pujos, Marc Trapman, Rogier Doornbos,
Pierre Van Cutsem, Rune Pedersen, Sergio Caceres, Hubertus Kleeberg, Arne Peters, Lucius Tamm
Improving biocontrol activity of Bacillus subtilis UTB1 against Aspergillus flavus using gamma-irradiation Hamideh Afsharmanesh, Masoud Ahmadzadeh, Abbas Majdabadi, Keyvan Behboodi, Mohammad Javan-Nikkhah, Farahnaz Motamedi, Houda Zeriouh, Diego Romero and Alejandro Perez-Garcia
Investigation of the volatile metabolome in conidiating and non-conidiating cultures of the filamentous fungus <i>Trichoderma virens</i> using HS-SPME-GC-MS <i>Alexandra Parich, Bernhard Kluger, Susanne Zeilinger, Prasun Mukherjee, Frankie Crutcher, Charles Kenerley, Rainer Schuhmacher</i>
Use of Isotope Ratio Mass Spectrometry (IRMS) for the study of trophic interaction among pathogen and antagonists Alberto Pellegrini, Paola Elisa Corneo, Federica Camin, Solveig Tosi, Ilaria Pertot
The SLU Centre for Biological Control: Generating new knowledge on sustainable application of biocontrol Ingvar Sundh, Hanna Friberg, Sebastian Håkansson, Mattias Jonsson, Margareta Hökeberg

Characterization and effect of bioactive compounds from luminescent mushroom,

Neonothopanus nambi Speg. on root-knot nematode

(Meloidogyne incognita Chitwood) of vegetables

Sureeporn Bua-art, Boossaracum Udomsak, Nuchanart Tangchitsomkid,

Abstract: Dry mycelium and culture filtrate from 3 isolates (PW1, PW2 and KKU) of luminescent mushroom (Neonothopanus nambi) were extracted in order to obtain bioactive compounds. Extraction using dry mycelium derived bioactive compound powder more than using culture filtrate with extraction efficiency of 8.7-12.9%. The effect of bioactive compound was investigated on infectious larvae (J2) of root-knot nematode (Meloidogyne incognita) in laboratory. The result showed that bioactive compound at concentration 500mg/l caused 100% mortality of J2 in 1 min. Concentrations of 100 and 50mg/l affected J2 causing significantly mortalities of 100% in 30 min and 48h, respectively. Effect of this bioactive compound on J2 was also confirmed in screened house experiment and found that concentrations of 100 and 500mg/l suppressed evidently J2 without root-knot symptom on tomato plants. Root-knot score 1 was detectable on tomato plants treated with the compound of 10 and 50mg/l. However, it was significantly (P < 0.05) from control treatment, which exhibited root-knot score 4. The mushroom spawn was also tested on control of root-knot nematode with chili plant under greenhouse condition. Spawn of 10, 20, 30, 40 and 40g/plant were infested in chili plants grown in pots. Galling percentage was evaluated on 30 days after treated with spawn. The result indicated that all rate of spawn suppressed root galling. In particular, the treatment of 10g/plant reduced significantly (P < 0.05) 85% (galling index 12%). While in control and chemical treatments, galling indexes were 75 and 60%, respectively. This study suggests the efficiency and potential of the application of bioactive compound from N. nambi for control root-knot nematode.

Potential biocontrol against sclerotial fungi using a mycoparasite,

Paraconiothyrium minitans CM2

Extended abstract

Priming for plant defence reactions in tomato roots

by the biocontrol strain Fusarium oxysporum 47

Sébastien Aimé, Claude Alabouvette, Christian Steinberg, Chantal Olivain 353-358

Abstract: An *in vitro* method enabling to inoculate tomato roots with Fusarium oxysporum 47 and 48h later with *Fusarium oxysporum* f. sp. *lycopersici* 8 was developed. Results show that colonization of tomato roots by Fo47 is able to induce a primed state of roots, which results in a higher expression level of defence-related genes (*GluA*, *Chi3*, *PR1a*) upon pathogenic *F. oxysporum* attack.

Effect of arabinogalactan proteins from the root caps of *Pisum sativum*

and Brassica napus on Aphanomyces euteiches zoospore

chemotaxis and germination

Marc Antoine Cannesan, Caroline Durand, Carole Burel,

Christophe Gangneux, Patrice Lerouge, Karine Laval,

Marie-Laure Follet-Gueye, Eric Nguema-Ona, Azeddine Driouich,

Extended abstract

Potential for biological control of plant pathogens by isolates from suppressive soils

F. Carretero, F. Diánez, F. Marín, M. A. Martínez, J. A. Yau,

Extended abstract

Animal origin wastes as new sources of resistance inducers against wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*)

Lubomir Vechet, Karel Kolomaznik, Jana Hanzalova, Nadezda Vrchotova 363-368

Abstract: Elicitors of animal origin were tested on wheat as inducers of resistance to powdery mildew. Their effect was compared with the inducer of chemical origin benzothiadiazole and with inducers of plant origin. Effect of animal- and plant-origin elicitors on disease severity differed in two years of experiments under field conditions. Inducers of animal origin could be potential sources of plant- induced resistance to diseases.