IOBC
OILB

WPRS International Organisation for Biological and Integrated Control of Noxious Animals and Plants: West Palaearctic Regional Section

SROP Organisation Internationale de Lutte Biologique et Integrée contre les Animaux et les Plantes Nuisibles: Section Régionale Ouest Paléarctique

WORKING GROUPS:
BIOLOGICAL CONTROL OF FUNGAL AND BACTERIAL PLANT PATHOGENS
INTEGRATED CONTROL IN PROTECTED CROPS, TEMPERATE CLIMATE

Integration 2004

IOBC

S. Michele all'Adige, Italy

Meeting of the WGs:

Management of plant diseases and arthropod pests by BCAs and their integration in agricultural systems

S. Michele all'Adige, Trentino, Italy
9-13 June 2004

PROGRAM and ABSTRACTS

Istituto Agrario di S. Michele all'Adige (IASMA), Italy
&
Centre for Research and Development of crop protection with low environment and consumer-health impact (SafeCrop)

Program and Abstracts

Management of plant diseases and arthropod pests by BCAs and their integration in agricultural systems

S. Michele all'Adige, Trentino, Italy
9-13 June 2004

Edited by:

Yigal Elad
Annie Enkegaard
Ilaria Pertot

IOBC/WPRS WORKING GROUPS:

BIOLOGICAL CONTROL OF FUNGAL AND BACTERIAL PLANT PATHOGENS

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In association with WG:

INTEGRATED CONTROL IN PROTECTED CROPS, MEDITERRANEAN CLIMATE

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Meeting Organization

Co-chairpersons:
Yigal Elad, Annie Enkegaard and Ilaria Pertot

Local organizing committee:
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Scientific Committee:
Annie Enkegaard, Yigal Elad, Justine Head, Sylvia Blümel, Claude Alabouvette, Lodovica Maria Gullino, Alberto Alma, Cristina Castañe, Ilaria Pertot, Diego Forti

International Advisory Scientific Committee:
Claude Alabouvette, Genéviève Défago, Yigal Elad, Dan Funk-Jensen, Jürgen Köhl, John M. Whipps

Local Organization

Hotel and Tourism: Trentino Holiday
Registration and general issues: Istituto Agrario di S. Michele all'Adige, TN

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Management of plant diseases and arthropod pests by BCAs and their integration in agricultural systems

S. Michele all'Adige, Trentino, Italy
9-13 June 2004

Introduction

Following the **meeting** of the Phytopathogens WG in **Kusadasi** 22-26 May 2002 and the meeting of the Temperate Climate WG in **Victoria, BC** 6-9 May 2002 and in cooperation with the Protected Crops Mediterranean WG, we organize an IOBC meeting in S. Michele, Trentino, Italy, at the foothills of the Dolomite mountains.

The theme chosen for workshop is: "Management of plant diseases and arthropod pests by BCAs and their integration in agricultural systems". The specific topics of the workshop are:

i. Integrated plant disease and arthropod pest management: possibilities for integration, problems with interactions between different tools of pest and disease management, positive and negative side effects on non-target organisms.
ii. Multi target agents, including both microbial and those derived from natural subtances, i.e. targeting several diseases or targeting disease (s) and pest (s).
iii. Side effects of arthropod pest management tools on disease development and control.
iv. Side effects of disease control on beneficials.
v. Case studies of implementation of integrated disease and pest management - successes and problems encountered.
vi. Integrated disease management.
vii. Integration of microbials and management of the greenhouse for IPM of pests and diseases according to decision support systems.
viii. Commercial use of microbials for pests and diseases management - present situation and prospects, including new/near registration products.
ix. Application of natural substances/microbials against diseases.
x. Role of host plant resistance in IPM of pests and diseases.
xi. Induced resistance towards diseases and pests.

A big number of participants from many countries participate in the meeting. Our aim is to encourage the discussion between entomologists, plant pathologists and other plant protection experts. In order to fill gaps that are evident in the field of true integrated disease and pest management, we allocated significant time to discussions on integration, holistic approaches and gaps in information and knowledge.

We wish you an interesting and fruitfull meeting in S. Michele all'Adige, Italy.

Sincerely,

Yigal Elad, Annie Enkegaard, Ilaria Pertot

Management of plant diseases and arthropod pests by BCAs and their integration in agricultural systems

MEETING PROGRAM

Wednesday 09.6.2004

Afternoon	Arrival of participants to Trento
18:00-20:30	Registration of participants in the hotel Accademia
18:30-20:30	Get together in the hotel Accademia

Thursday 10.6.2004

08:30-10:00 **Opening Session**
Chair persons: Ilaria Pertot, Yigal Elad, Annie Enkegaard

Speakers:
Dr. Gianluca Salvatori, assessore programmazione, ricerca e innovazione
Dr. Alessandro Dini, general manager of Istituto Agrario di S. Michele all'Adige
Dr. Cesare Gessler, scientific director of SafeCrop Centre
Dr. Yigal Elad, convenor of IOBC/WPRS WG Biological control of fungal and bacterial plant pathogens

10:00-10:30 Tea/Coffee Break

10:30-13:00 Oral Session 1: Combined management for control of pests and diseases
Chairpersons: Cristina Castañé and Gabriele Berg

Marc Bardin, Jacques Fargues, Laurent Couston, Claire Troulet, Géraldine Philippe, Philippe C. Nicot	Compatibility of three biological methods to control grey mould, powdery mildew and whitefly on tomato	20
Derridj Sylvie, Borges Alexis	Incidence of application of an elicitor of apple tree resistance against fire blight (*Erwinia amylovora*) on an insect pest codling moth (*Cydia pomonella*) *Lepidoptera*, *Tortricidae* egg laying	21
Alon Bilu, Dalia Rav David, Arnon Dag, Sharoni Shafir, Mohamad Abu-Toamy, Yigal Elad	Using honeybees to deliver a biocontrol agent for the control of strawberry *Botrytis cinerea*-fruit rots	22
Sonia Ganassi, Antonio Logrieco, Antonio De Cristofaro, Maria Agnese Sabatini	Laboratory evaluation of antifeedant activity of *Trichoderma* spp. isolates in aphid biocontrol	23
Giorgio Maresi, Gino Angeli, Tullio Turchetti	Biological control in chestnut cultivation: criteria for a sustainable management	24
Aleid Dik, Dirk Jan van der Gaag, Juliette Pijnakker, Pim Paternotte, Jos Wubben	Development of control strategies and implementation by growers	25
Masanori Koike, Toshiki Higashio, Akio Komori, Kyouko Akiyama, Noriko Kishimoto, Emi Masuda, Mai Sasaki, Sanae Yoshida, Masayuki Tani, Katsuhisa Kuramoti, Midori Sugimoto, Hideyuki Nagao	*Verticillium lecanii* (*Lecanicillium* spp.) as epiphyte and their application to biological control of pest and disease in a glasshouse and a field	26
Walt Bentley, Shawn Steffan, Scott Johnson, Gary Vansickle	Implementation of an arthropod pest management program for peaches and plums where organophosphate and carbamate sprays are eliminated	27

13:00-14:00 Lunch Break

14:00-14:45 Oral Session 2a: Integrated control of diseases
Chairperson: John Whipps

Neta Okon Levy, Yigal Elad, Jaacov Katan	Integration of *Trichoderma* and soil solarization for disease management	29
John P. Clarkson, Anita Scruby, Emma Coventry, Ralph Noble, John M. Whipps	Integrated control of *Allium* white rot using biological control agents, composted onion waste and tebuconazole treated seed	30
Roberta Roberti, Federica Badiali, Annamaria Pisi, Annarita Veronesi, Augusto Cesari	Sensitivity of *Clonostachys rosea* and different strains of *Trichoderma* to fungicides and herbicides	31

14:45-15:30 Oral Session 2b: Risk characterization of BCAs
Chairperson: Luciana Corazza

15:30-16:00 Discussion Session 2a and 2b

16:00-17:00 Poster Session 1

Leszek B. Orlikowski	Grapefruit extract inhibits sporulation and development of *Phytophthora ramorum* on rhododendron	p1-51	87
Leszek B. Orlikowski	Control of *Phytophthora cryptogea* with *Trichoderma viride* combined with furalaxyl and chitosan	p1-53	88
Valdebenito-Sanhueza Rosa.Maria, Guimaraes Leticia, Camatti-Sartori Valdirene, Ribeiro Rute Terezinha da Silva	Survey of antagonistic yeasts occurring in apple trees managed with different production systems	p1-55	89
Marjan de Boer, Rik de Werd, Vincent Bijman, Suzanne Breeuwsmaand, Jos Raaijmakers	Integrated management of *Pythium* root rot in flower bulb production	p1-57	90
Dimakopoulou Myrto, Tjamos Eleftherios C., Antoniou Polymnia, Tjamos S. E.	Laboratory and field evaluation of epiphytic vine yeasts against sour rot of grapes caused by *Aspergillus carbonarius*	p1-59	91
Ragni Adriano, Quattrocchi Laura, Coranelli Simona, Manuele Ricci	Selection of entomopathogenic nematodes for heat tolerant and desiccation traits	p1-61	92
Davide Spadaro, Angelo Garibaldi, Maria Lodovica Gullino	Integrated control of blue mould on apple with biocontrol agents, chemical alternatives and heat treatment	p1-63	93
Nicola La Porta, Renata Grillo, Paolo Ambrosi, Ari M. Hietala	Comparison of wood colonisation by Alpine *Phlebia gigantea* strains, Rotstop® and *Trichoderma* treatments in a pure Norway spruce stand in Alpine environment	p1-65	94
Nicola La Porta, Renata Grillo, Paolo Ambrosi, Kari Korhonen	*In vitro* antagonism of *Armillaria* spp. against *Heterobasidion annosum* s.l.	p1-67	95
Francesco Savino, Andrea Iodice, Vittorio Veronelli	Selectivity and effectiveness evaluation of Electrolyzed Acid Water (EAW), alone and in mixture with Shin-Etsu surfactants, against *Phytophthora infestans* (Mont.) De Bary, the causal organism of late blight on tomato	p1-69	96

15:30-17:30 During poster sessionTea/Coffee served

17:15-18:15 Poster Session 2

Henry Müller, Remo Meincke, Gabriele Berg	*Serratia plymuthica* HRO-C48: Strategy to control *Verticillium dahliae* in oilseed rape and effects on the microbial community	p2-2	98
Francisco M. Cazorla, Diego J. Ruíz-Romero, Eva Arrebola, Guido V. Bloemberg, Alejandro Pérez-García, Ben J.J. Lugtenberg, Antonio de Vicente	Biocontrol of avocado white root rot by using the bacterial strain *Pseudomonas fluorescens* pcl1606	p2-4	99

Tjamos Eleftherios C., Panorios Dimitrios G., Polissiou Moschos G., Daferera Dimitra J., Alissandrakis Eleftherios, Dimakopoulou Myrto	Evaluation methodologies and mode of action of epiphytic vine yeasts against *Botrytis cinerea* of grapes	p2-62	128
Ricci Manuele, Fifi Anna Paola, De Berardinis Marta, Colli Monica, Barcarotti Rosita, Ragni Adriano	Development of a bio-inseticide based on a cold-active entomopathogenic nematode	p2-64	129
Nicola Sante Iacobellis, Pietro Lo Cantore, Adriana De Marco, Francesco Capasso, Felice Senatore	Antibacterial activity of essential oils	p2-66	130
Kerstin Jung	Combined use of insectpathogenic fungi and nematodes against the onion thrips, *Thrips tabaci* in the field	p2-68	131

18:15-19:30 Mini excursion (visit to the “Cantine Mezzacorona” vinery and wine testing)

Evening Dinner in “Birreria” in Trento (**please register** at registration desk before 12.00) or free

Friday 11.6.2004

08:00-10:00 Mini excursion to strawberry and small fruits greenhouse tunnels

10:00-10:30 Coffee Break

10:30-12:30 Round Table: What will be the future for BCAs?

Moderator:
Cesare Gessler

Participants:

Elzbieta-Barbara Ceglarska: EU policies in biocontrol research and biocontrol implementation.
Cesare Gessler: Risk related to BCAs: reality or phantom risk?
Aleid Dik: Experience and problems in transferring scientific results into practice
Jürg Huber: Future directions for improvement of research and development of BCAs
Sergio Franceschini: EU registration problems and possible solution
Massimo Benuzzi: The industry point of view on problems in developing BCAs

12:30-evening Excursion (visit to apple orchards and “Melinda” Growers association) and dinner

Saturday 12.6.2004

08:30-10:00 Oral Session 3: Management of soil borne diseases
Chairpersons: Lodovica Gullino and Joeke Postma

Emma Coventry, Léon Fayolle, Sebastien Aimé, Claude Alabouvette, Ralph Noble, John Whipps	Eradication of plant pathogens and pests from composting wastes and their use in disease suppression	37
Martin Kirchmair, Lars Huber, Hermann Strasser	The use of *Metarhizium anisopliae* against grape phylloxera: first results	38
Inge M.B. Knudsen, Birgit Jensen, Mette Lübeck, Dan Funck Jensen	Multi-target Biocontrol Efficacy of *Clonostachys rosea* IK726	39
Wilfred Otten, Anne Bates, Christopher A. Gilligan	Analysis of efficacy of a biocontrol agent to reduce the transmission of infection in damping-off epidemics	40
John M. Whipps, Amanda J. Bennett, Michael P. Challen, Robert A. Hill, Daohong Jiang, E.Eirian Jones, Mark P. McQuilken, Arjen Rinzema, Christopher W. Rogers, Alison Stewart, Nicola E. Tomprefa	Recent developments in inoculum production, application and pathogenicity in the biocontrol agent *Coniothyrium minitans*	41
Isabel Grondona, Alfonso Rodríguez, Martha I. Gómez, Rafael Camacho, Antonio Llobell, Enrique Monte	TUSAL®, a commercial biocontrol formulation based on *Trichoderma*	42

10:00-10:30 Discussion Session 3

10:30-11:00 Tea/Coffee Break

11:00-11:45 Oral Session 4a: Mode of action of biocontrol agents
Chairperson: Enrique Monte

Gabriele Berg, Annette Krechel, Franziska Faltin, Andreas Ulrich, Johannes Hallmann, Rita Grosch	Endophytes: a new source for multi-target biological control agents?	44
Sheridan L. Woo, Michelina Ruocco, Patrizia Ambrosino, Roberta Marra, Rosalia Ciliento, Stefania Lanzuise, Valeria Scala, Francesco Vinale, Sara Gigante, Lucia Catapano, Felice Scala, Matteo Lorito	Novel understanding of the biocontrol mechanisms of *Trichoderma*, a mycoparasite and an opportunistic avirulent plant symbiont	45
Wolfgang Zeller, Kamal Ahmed Mohamed Abo-Elyousr	Studies on induced resistance against fire blight (*Erwinia amylovora*) with different bioagents	46

11:45-12:30 Oral Session 4b: Postharvest
Chairperson: Haïssam Jijakli

Sagi Gavriel, Zafar Ismailov, Marianna Ovadis, Ilan Chet Leonid Chernin	Comparative study of the role of chitinase and pyrrolnitrin for biocontrol activity of *Serratia plymuthica* IC1270	48
Stella Mokiou, Naresh Magan	Formulation and shelf-life studies of the biocontrol yeast *Pichia anomala*: positive effect of endogenous solutes, isotonic solutions and additives during fluidised-bed drying	49
Anna Bonaterra, Jesús M. Francés, M. Carmen Moreno, Esther Badosa, Emilio Montesinos	Postharvest biological control of a wide range of pathogens and fruit types by *Pantoea agglomerans* EPS125	50

12:30-13:00 Discussion Sessions 4a and 4b

13:00-13:50 Lunch Break

13:50-14:00 Group Picture (meeting point: main entrance of the Institute)

14:00-15:00 Oral Session 5a: Combination of control means
Chairpersons: Dani Shtienberg and Jürgen Köhl

Jürgen Köhl, Bert Evenhuis, Pedro Boff	Integration of the use of the antagonist *Ulocladium atrum* in management of strawberry grey mould (*Botrytis cinerea*)	52
Graziella Berta, Simonetta Sampo, Elisa Gamalero, Nadia Massa, Philippe Lemanceau	Suppression of *Rhizoctonia* root rot of tomato by *Glomus mosseae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with combined modes of action	53
Joeke Postma, Margarit Willemsen-de Klein	Biological control of *Pythium aphanidermatum* in cucumber with combined applications of bacterial antagonists with chitosan	54
Raffaelli Roberta, Riccarda Moser, Ilaria Pertot	Evaluation of the sustainability of strategies that include biocontrol agents to reduce chemical residues on strawberry fruits	55

15:00-16:00 **Oral Session 5b: Integrated management of diseases**
Chairpersons: Aleid Dik and Elzbieta-Barbara Ceglarska

Ilaria Pertot, Rosaly Zasso, Liat Amsalem, Mario Baldessari, Gino Angeli, Yigal Elad	Use of biocontrol agents against powdery mildew in integrated strategies for reducing pesticide residues on strawberry: efficacy and side effects evaluation	57
Dani Shtienberg, Haim Vintal, Miri Targerman, Yoel Mesika, Uri Adler, Eli Matan, Yigal Elad	Integrated management of late blight in greenhouse tomatoes	58
Manochehr Azarang, Lennart Johnsson, Berndt Gerhardson, David B. Collinge, Sandra Wright	An integrated approach to simultaneously control insect pests, powdery mildew and seed borne fungal diseases in barley by bacterial seed treatment	59
Marjan de Boer, Rik de Werd, Ineke Pennock-Vos, Joop van Doorn, Ernst van den Ende	Development of a management system for integrated and biological control of *Botrytis* spp. in flower bulb crops	60

16:00-16:30 **Discussion Sessions**

16:30-18:30 **General discussion (Tea/Coffee served)**
Moderators: Justine Head, Yigal Elad, Annie Enkegaard

21:00 Meeting Dinner (at Castel Toblino)

Sunday 13.6.2004

Departure of participants

ABSTRACTS

Oral session 1: Combined management for control of pests and diseases

Compatibility of three biological methods to control grey mould, powdery mildew and whitefly on tomato

Marc Bardin[1], Jacques Fargues[2], Laurent Couston[1,2], Claire Troulet[1], Géraldine Philippe[1], Philippe C. Nicot[1]

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Grey mould caused by *Botrytis cinerea* and powdery mildew caused by the recently renamed species *Oidium neolycopersici* are among the most important diseases encountered on tomato glasshouses. In addition, the whitefly *Bemisia tabaci* creates increasing problems in Mediterranean countries in many different greenhouse crops including tomato. Methods to control these bio-aggressors are mainly based on chemical treatments. Their insufficient efficacy and their negative effect in integrated control programs have encouraged the development of alternative methods of control. Efficient biological control agents have been selected to protect tomato against *B. cinerea*. Among them, strain L13 of *Microdochium dimerum* has shown a high efficiency to protect pruning wounds against *B. cinerea* at crop level. Various biological methods have been reported to control powdery mildews. A plant extract from the giant knotweed *Reynoutria sachalinensis* (trade name Milsana) has shown a high efficiency to control powdery mildews on tomato. The compatibility between this plant extract and *M. dimerum* suggests that they could be used in alternation or in combination together. Various hyphomycetes have been selected for their potential as mycoinsecticides for whitefly control. Among them, *Lecanicillium lecanii* [*Verticillium lecanii*], trade name Mycotal, appears to be a promising fungal biocontrol agent.

The use of the two biological control agents, *M. dimerum* and *L. lecanii,* and the plant extract Milsana in a context of integrated protection on tomato requires that their efficiency is not altered when applied in conjunction or in alternation. The purpose of the present study was to evaluate the compatibility of *M. dimerum* and Milsana with *L. lecanii* and vice versa. To this end, the effect of the application of *L. lecanii* (1) on the susceptibility of tomato to *B. cinerea* and *O. neolycopersici* and (2) on the efficacy of *M. dimerum* and Milsana towards *B. cinerea* and *O. neolycopersici* was evaluated. Reciprocally, the effect of the application of *M. dimerum* and Milsana (1) on the susceptibility of tomato to infestation by *B. tabaci* and (2) on the efficacy of *L. lecanii* towards *B. tabaci* was estimated. Compatibility tests were conducted on potted plants in controlled conditions. Mycotal had no significant effect on the susceptibility of tomato to *B. cinerea* and *O. neolycopersici* and *M. dimerum* and Milsana had no effect on the susceptibility of tomato to infestation by *B. tabaci*. Moreover, the efficacy of Mycotal was not altered by application of *M. dimerum* or Milsana and the efficacy of *M. dimerum* and Milsana was not altered by application of Mycotal. These results suggest that *M. dimerum*, Milsana and Mycotal have potential to be included into an integrated protection scheme of greenhouse tomatoes.

Incidence of application of an elicitor of apple tree resistance against fire blight (*Erwinia amylovora*) on an insect pest codling moth (*Cydia pomonella) Lepidoptera, Tortricidae* egg laying

Derridj Sylvie, Borges Alexis

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The use of systemic acquired resistance (SAR) as a component in an orchard ecologically based management is expected as a potential tool of control against the bacteria *Erwinia amylovora*. The aim of our study was to look at eventual consequences of the use of acibenzolar-S-methyl (ASM), on egg laying of *Cydia pomonella,* the major insect pest of apple tree. We already demonstrated that acceptance of plant by the insect and its egg laying are influenced by metabolites, which are present on the plant surface. In the frame of this study we looked at these metabolites.

ASM was sprayed on apple trees on the 3rd and 6th of June 2003 at the concentration of 100 mg per litter of the active product. Trees were 3 years old, 190 cm height, cultivated in containers in the open. Mass rearing insects were released on trees from 11 to 19 days after the treatment. 40 females were released per tree in screen cages in no choice conditions (4 replicates per treatment: non treated and ASM treated plant). Counting of alighting and eggs were carried on from 16h to 17h solar time just after insect release in cages. Collect of plant surface water washings on the under and upper sides of different types of leaves and apple were achieved at the end of insect observations. We analysed 7 primary metabolites from the washings. Artificial substrates were then impregnated with the analysed metabolites reproducing the composition of the upper leaf side of young shoot of treated and non-treated plants and given to females for egg laying in no choice condition (30 replicates). The treatment of trees with ASM reduced by 60% the number of eggs per tree: 27.0 ± 8 against 67.5 ± 14.6 on non-treated trees. The distribution of eggs within the tree was not changed by the treatment, on corymb, shoot leaf and other leaves and apple, proportions of eggs were respectively 12, 43, 23 and 22%. Egg number was mainly reduced on the upper leaf side of the shoot by 70%, 5.75 ±2.2 vs. 21.0 ± 4.8 on non-treated plants. On the treated trees the number of females alighting from the cage walls to the tree was reduced by 50% vs. non-treated trees. Whereas within the tree the numbers of alighting from site to site were similar in both treatments. Chemical analyses of metabolites of plant surface washings did not show any difference between the treatments according to the plant sites except on the upper leaf side of the shoots. On them when treated there were lower quantities of glucose, sucrose, mannitol and myo-inositol: 3.13±0.73, 7.39±1.22, 1.96±0.94, 2.74±0.92 ng per cm² respectively vs. 7.84 ±1.39, 31.38±7.66, 3.62 ±3.62+0.83, 7.59±1.36 ng per cm² on non-treated shoot leaves.

Females accepted in the same way artificial substrates impregnated with the 7 metabolites reproducing the treated and non-treated shoot leaf composition. Among similar number groups of female which laid ≤ 10 eggs, egg per female was 2.6 ± 0.6 on the treated shoot leaf composition vs. 6 ±1.51 eggs on non treated shoot leaf composition. The use of ASM diminished *C. pomonella* egg numbers on trees. It could be explained by a reduction of the insect alighting on trees and by a lower egg laying stimulation by contact. For this last point this could be explained by the decrease of quantities of metabolites known as egg laying stimulant.

Using honeybees to deliver a biocontrol agent for the control of strawberry *Botrytis cinerea*-fruit rots

Alon Bilu[1,3], Dalia Rav David[1], Arnon Dag[2], Sharoni Shafir[3], Mohamad Abu-Toamy[4], Yigal Elad[1]

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The ability of honey bees and bumblebees to vector microbial agents can be harnessed for biological control purposes, by using them to transfer inoculum of fungi and bacteria from the hive to flowers. The present study involved the biocontrol preparation TRICHODEX (*Trichoderma harzianum* T39) and honey bees for treating flowers of strawberry plants against the fungal pathogen *Botrytis cinerea* infection and development of flower and fruit grey mould. Biocontrol of strawberry fruit grey mould by spraying the biocontrol preparation is ineffective unless very frequent applications are done. Initially we evaluated the relative efficiency of four powder dispensers in loading honeybees with the *T. harzianum* T39 preparation. The newly developed 'Triwaks' dispenser was found more effective than three other dispenser types, 'Peng', 'Tub' and 'Harwood', with respect to effects of the dispensers on bee activity and effectiveness of contaminating bees with high levels of *Trichoderma* inoculum for a long period of time after its application. We found differences in these parameters of performance between dispenser types, with the Triwaks dispenser having the overall best performance. Bees leaving hives equipped with the Triwaks dispenser were loaded at the most with $1.45*10^5$ cfu *T. harzianum* T39/bee. This dispenser was further tested to evaluate the population level of *T. harzianum* T39 on flowers visited by the bees. Strawberry flowers in a commercial polyethylene greenhouse located 24 meter far from hives with *Trichoderma* loaded dispenser had $3.6*10^3$ cfu/flower. Similar level of *Trichoderma* was found on flowers immediately after *Trichoderma* spray, but the level declined with time. These levels were found necessary for the control of strawberry grey mould. Thus, bees proved to be a potential means for the delivery of the biocontrol fungus to the infection site of *B. cinerea.* Ten beehives were placed at the edge of a commercial strawberry field and the biocontrol preparation TRICHODEX was added to their dispensers on a daily basis from mid December until mid March. Experimental plots were located 25-50 m from the hives. During the period of grey mould development, bee delivered *T. harzianum* T39 was found to be as good as or somewhat better than season long chemical treatment (fludioxonil+cyprodinil and pyrimethanil). It can be concluded that the delivery of *Trichoderma* by bees to strawberry flowers is effective in strawberry fruit grey mould control and can be integrated in an IPM scheme in this crop.

Laboratory evaluation of antifeedant activity of *Trichoderma* spp. isolates in aphid biocontrol

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Fungi offer several potential strategies against pest insects to reduce crop losses. Indeed, fungi and their metabolites can influence, by different modalities, several traits of the insect biology, such as survival, development, growth, fecundity and feeding behaviour. Nonetheless, in general, very few data are available on the repellency of fungi towards insect pests. We started a laboratory study aimed at evaluating the potential of secondary metabolites synthesized by fungal isolates belonging to different species of the genus *Trichoderma* (Deuteromycetes) as antifeedant compounds towards phytophagous insects such as aphids. The final goal aimed to understand the mechanism behind the capacity of aphids to detect certain metabolites and walk off. Repellency offers an alternative management strategy interfering with aphid processes of host plant selection and feeding behaviour and may lead to a reduction of both direct feeding damage and virus transmission. Tests were carried out utilising three species of aphids (*Homoptera: Aphididae*): *Schizaphis graminum* (Rondani), one of the most important pest of cereal crops; *Megoura viciae* (Buckton), an insect pest of leguminous crops; and *Myzus persicae* (Sulzer), a cosmopolitan species that attacks plants in the field, readily infests vegetables and ornamental plants grown in greenhouses, and is highly noxious due to its ability to transmit plant viruses. The fungi isolates were administered to aphids in the form of a fungal culture that was cultured on rice kernels and than dried and finely ground. Trials carried out utilising adult aphids pointed out an antifeedant activity of some isolates belonging to different species of the genus *Trichoderma* against aphids of the three species. A repellent effect was detected over the entire duration of the trials lasting 9 hours. However, a different sensitivity was observed across the winged and wingless morphs of *S. graminum* towards the fungal cultures applied on leaves of the host plant. Indeed, some isolates were repellent for winged but not for wingless morphs. In terms of the sensory organs involved in the perception of metabolites, electroantennographic studies, carried out with two species, *S. graminum* and *M. viciae*, showed that volatile compounds of fungal cultures were not able to stimulate the olfactory sensilla of antennae of both aphid species; as a consequence these substances cannot be responsible for the repellent effect. Taste sensilla lodged on the mesothoracic distal tarsomere of both aphid species were more strongly stimulated by aqueous solutions of fungal cultures than aqueous solutions containing the rice kernels finely ground alone. The observed aphid behaviour might be due to their sensitivity to water soluble compounds, with a repellent activity, produced by *Trichoderma* isolates.

Biological control in chestnut cultivation: criteria for a sustainable management

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Chestnut stands are still an important resource for European mountain regions either for fruit and wood production or for landscape and recreational value. Diseases and pests can severely affect cultivation but their control needs to respect the ecological equilibrium of this stands, which are mainly forest situations. In the last years researches carried out in Italy and in other European country were focused to define criteria to obtain a good biological control of the main phytosanitary problems: chestnut blight, ink diseases and cydia damages. The natural spread of hypovirulent strains of *Cryphonectria parasitica* has enhanced the spontaneous recovering of many chestnut stands and now selvicoltural management, artificial inoculation and biological wax for grafts protection are available to maintain or increase the hypovirulence effectiveness. Soil management by means of manuring proved to be useful in the control of ink disease foci, aiming to restore natural competitors to *Phytophthorae.* Pheromones for cydiae monitoring were improved recently and techniques of mating disruption are going to be tested. The possibility to have cheap and effective biological control methods is needed to increase chestnut cultivation sustainability.

Development of control strategies and implementation by growers

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Control strategies - A lot of knowledge on integrated and biological crop protection is available for a number of important glasshouse crops. Much of this knowledge is obtained in research on solutions against a particular pest or disease. In order to develop cropping systems with good integrated or biological control, all available knowledge has to be used simultaneously in an optimum way. Applied Plant Research has started several new projects to reach this goal: *i.* inventory of all known methods and compounds and natural enemies against all pests and diseases in economically important glasshouse vegetables and floriculture crops, *ii.* research on interaction between biological and natural compounds, *iii.* testing of all integrated and biological control strategies simultaneously in the most important glasshouse crops. The inventory is finished and in the research on testing interactions practically all known effective biological and natural products have been tested. The results are used in the testing of control strategies, in which the best combinations of crop protection measures and compounds against all diseases and pests are sought. In cucumber, chrysanthemum and rose, several strategies have been compared. Currently, an experiment is run in tomato in which the interaction between energy input and crop protection is also included. The results show that the harmful impact on the environment can be reduced by integration of guided chemical control, cropping methods (glasshouse climate, choice of cultivar, pruning method etcetera), biological control and natural products, while at the same time maintaining a high production level. The best strategies are now implemented in commercial glasshouses in grower groups co-ordinated by researchers.

Grower groups - Recently, a four-year project has started with the goal to help growers with implementation of integrated control strategies. Groups of growers have been formed for cucumber, tomato, rose and chrysanthemum. Each group consists of five to ten growers and a researcher from Applied Plant Research – Horticulture. Each grower makes a crop protection plan for his crop, together with the researcher and his regular adviser from a crop protection company. In this plan, environmental impact and economic factors will be included. During the cropping period, total input of time, compounds and natural enemies are registered as well as harvested product. At the end, the results are analysed for environmental impact and financial result. With this analysis, a crop protection strategy for the next crop is developed. The goal is to implement more and more integrated control measures. The results from the aforementioned projects are included in this implementation project. By elaborate communication of strategies and results, we hope to convince more and more growers to implement reliable and economically sound integrated and biological control strategies.

Verticillium lecanii (*Lecanicillium* spp.) as epiphyte and their application to biological control of pest and disease in a glasshouse and a field

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Twenty strains of *Verticillium lecanii* (*Lecanicillium* spp.) isolated from plant pathogenic fungi, aphids and whiteflies, including 2 commercialized strains (Mycotal and Vertalec) were tested for their ability to colonize leaf surfaces of cucumber, strawberry, tomato and wheat grown in a glasshouse. Leaf surfaces were inoculated with *V. lecanii* by spraying spore suspensions (1×10^7 /ml/leaf) of each strain. The abilities of colonization on the leaf surfaces were confirmed by dilution plate method. Three strains (A-2, B-2 and C-1) showed higher ability of colonization at 2 and 4 weeks after inoculation compared to other strains. These strains were studied to determine their potential in epiphytic biocontrol of whitefly (on cucumber and tomato), aphids (on tomato) and powdery mildew (on cucumber and melon). Data from glasshouse studies indicated that these three epiphytic strains, especially B-2 inoculation to leaves of tomato and cucumber reduced not only population growth of whitefly (on tomato, cucumber and melon) and aphids (on tomato, cucumber and melon) but also disease symptoms of powdery mildew on cucumber and melon. Furthermore, in a strawberry field trial, three strains (A-2, B-2 and C-1) and pesticides were also examined for side effect against carabid and flower-visiting insects diptera and hymenoptera. The pesticides killed all carabid under treatment but three isolated had very little impact on carabid and flower-visiting insects as much as control. Hence, B-2 may be a better candidate to take forward for development as a microbial control agent.

Implementation of an arthropod pest management program for peaches and plums where organophosphate and carbamate sprays are eliminated

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Pest management in fresh market stone fruit production in the United States has relied on organophosphate and carbamate insecticides to reduce arthropod pest damage since the development of these pesticides in the 1940's. Although quite effective, environmental problems and consumer concerns have arisen. Beginning in 1999, a program to manage arthropod pests in peach, nectarine, and plum without these pesticides was initiated. This program relied on mating disruption for Oriental fruit moth *Grapholitha molesta*, horticultural mineral oil application for San Jose scale *Diaspidiotus pernicious* and European red mite *Panonychus ulmi*, bloom time applications of *Bacillus thuringiensis* or spinosad for peach twig borer *Anarsia lineatella*, and summer sprays of spinosad for forked tailed bush katydid *Scudderia furcata* and omnivorous leaf roller *Platynota stultana.* Ten farms were selected whereby orchards of the same variety compared the above management approach (Reduced Risk) with the Conventional approach. The Conventional approach utilized the standard organophosphate and carbamate pesticides for managing key pests. The orchards were intensively monitored each week from February through September. Orchards were located in five peach producing counties in California's San Joaquin Valley.

The results of the study showed no significant (P>0.05, Fisher's Protected LSD) difference in pest damage and no difference in grower costs between the two approaches to pest management. The four-year average of insect damage for the Conventional and the Reduced Risk orchards was 8.05% and 8.24% respectively. Key pests such as Oriental fruit moth, San Jose scale, and Peach twig borer averaged less than 1% each year. The greatest damage occurred from forked tail bush katydid, a recently recognized pest. The four-year average katydid damage was 3.25% and 3.21% for the Conventional and Reduced Risk Programs. The cost per acre averaged $188 for each program.

Oral Session 2a: Integrated control of diseases

Integration of *Trichoderma* and soil solarization for disease management

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Soil solarization and *Trichoderma* application are used to suppress pathogens and have a positive effect on plant growth and yield. We investigated the effects of solarization and *T. harzianum* T39 soil treatments, alone or combined, on yield and on disease suppression, especially when a plant growth medium was reused. *Trichoderma* was applied after solarization. Reusing the substrate growth medium ('old medium') reduced yield, but the above treatments counteracted the negative effects of the 'old medium'. Trichoderma application to soil, solarization or combination of both treatments were as effective as using a new medium, thus enabling reusing the growth media. Strawberry, cucumber and beans grown on treated 'old' soil or growth substrate showed significant reduction in disease after leaf inoculation with *Botrytis cinerea* (grey mould) or with *Sphaerotheca fuliginea* (powdery mildew) on cucumber, hence indicating induced resistance. No significant changes in culturable microorganism populations, except for increased population levels of bacteria in rhizosphere of plants treated with the Trichoderma, were detected when using the method of dilution on selective agar media for microorganism enumeration. Attempts are being made to use a molecular technique based on 16S ribosomal DNA fingerprints. Denaturant gradient gel electrophoresis (DGGE) reveals different band patterns of rhizosphere populations of the different treatments.

Integrated control of *Allium* white rot using biological control agents, composted onion waste and tebuconazole treated seed

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Allium white rot (AWR) caused by the soil-borne fungus, Sclerotium cepivorum, is a major problem for the onion-growing industry worldwide. In the UK, large areas of onion-growing soils have been lost due to AWR infestation and chemical control is limited to tebuconazole treated seed. There is, therefore, an increasing need for alternative and sustainable methods of controlling AWR. The aim of this project was to investigate the potential of combining biological control agents (BCAs) with composted onion waste or tebuconazole seed treatments in an integrated system. Composted onion waste has previously been shown to reduce AWR in glasshouse tests. Two isolates of *Trichoderma viride* were found to be effective fungal BCAs of *S. cepivorum*. In laboratory tests where BCAs were added as wheat bran cultures to S. cepivorum sclerotia in soil, between 80 and 90% of the sclerotia were degraded after 8 weeks at 20°C. In a glasshouse bioassay where BCAs were added to soil infested with sclerotia and onion seed planted, AWR was reduced by at least 50% for three commercial onion varieties. Similar standard glasshouse bioassays were also used to test the efficacy of BCAs when combined with either composted onion waste (50% w/w in soil) or tebuconzole seed treatments (cv. White Lisbon). Generally it was found that the control of AWR was better in the combination treatments than with any treatment alone and up to 80-90% control was achieved.

Combinations of BCAs, composted onion waste and tebuconazole seed treatments therefore have great potential as an integrated system for AWR control in the UK. Enhancing the performance of BCAs is important as often the level of control by BCAs alone is not acceptable to growers. Further work will now test the efficacy of an integrated control programme in the field.

Sensitivity of *Clonostachys rosea* and different strains of *Trichoderma* to fungicides and herbicides

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Some selected antagonistic fungi, *Clonostachys rosea* 47 (CR47), *Trichoderma atroviride* 59 (TA59), *T. atroviride* 312 (TA312), *T. harzianum* 24 (TH24), *T. longibrachiatum* 9 (TL9), *T. longibrachiatum* 144 (TL144) and *T. viride* 15 (TV15), were tested to evaluate their *in vitro* sensitivity, as colony growth and conidial germination, towards five fungicides (carboxin, guazatine, prochloraz, thiram and triticonazole) and four herbicides (chlorsulfuron, chlortoluron, flufenacet and pendimethalin). Various degrees of sensitivity between antagonistic fungi and fungicides or herbicides were found. All antagonists showed low sensitivity to carboxin and thiram, and high sensitivity to prochloraz. More specifically, for mycelial radial growth, TV15 was almost insensitive to carboxin and thiram and highly sensitive to guazatine (EC_{50} 1.80 μg ml^{-1}), prochloraz (0.10 μg ml^{-1}) and triticonazole (2.83 μg ml^{-1}), TH24 was quite insensitive to carboxin (167.71 μg ml^{-1}), guazatine (204.84 μg ml^{-1}) and thiram (951.06 μg ml^{-1}). For conidial germination, TL144 was the most sensitive except for carboxin (EC_{50} not detectable). CR47, for either mycelial radial growth or conidial germination, was the least sensitive to fungicide treatments, with the exception of prochloraz (3.79 μg ml^{-1} and 0.01 μg ml^{-1} respectively). All the antagonists were almost insensitive to the tested herbicides, applied at field dose, and their mycelial radial growth was differently stimulated, except for TL144, which was slightly inhibited in presence of chlorotoluron. Herbicides stimulated the germ-tube growth of most antagonists, except for TA312, chlorotoluron and flufenacet induced a slight reduction of TL9 and TL144 hyphal elongation. The *in vitro* toxicity of prochloraz, guazatine and triticonazole towards the selected antagonists and the low sensitivity to the tested herbicides were confirmed by light (LM) and scanning electron microscope (SEM). Most of the treated sensitive fungi observed, showed at LM absence of conidia and hyphal disruptions and at SEM damaged hyphae with cell wall depression, degeneration and extrusion of cytoplasm content.

Oral Session 2b: Risk characterization of BCAs

Risk assessment of soil-applied fungal biological control agents with respect to European registration requirements

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Entomopathogenic fungi have significant potential as biological control agents (BCAs) against subterranean insect pests. For example, *Beauveria brongniartii* (Ascomycota, anamorph of Clavicipitales) is already used against *Melolontha melolontha* larvae (Coleoptera, Scarabaeidae) in Austria, Italy, Switzerland and France. However, a responsible risk assessment of fungal biological control agents is necessary from an ecological perspective. As part of the RAFBCA project, supported by the European Commission, Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 on Food, Nutrition and Health (Contract n°QLK1-CT-2001-01391), an extensive three year field trial with Beauveria brongniartii is carried out on the facilities of the GLP/GEP-certified ATC-Agro Trial Center GmbH in Burgenland, Austria. The experiments were designed to (1) study possible phytotoxic effects, (2) determine if relevant fungal metabolites enter the food chain and (3) monitor the persistence and possible dispersal of the BCA.

Field trials conducted with *Metarhizium anisopliae* include efficacy studies against the garden chafer (*Phyllopertha horticola*) and preliminary experiments for the evaluation of its potential to control grape phylloxera (*Dactulosphaira vitifoliae*). Soil samples collected in these studies are analysed for their content of *B. brongniartii* and *Metarhizium anisopliae*, respectively, to gain new information and complement existing data on the behaviour of these BCAs in the environment. The levels of BCA in the soil are compared with data on the diversity and abundance of common soil fungi such as *Trichoderma, Fusarium, Paecilomyces, Gliocladium, Penicillium, Aspergillus* and *Zygomycetes*. In order to complete the picture, the bacterial community is characterised by „Community Level Physiological Profiling“ using the Biolog® system. The collected data is correlated to detect possible effects of the introduction of BCAs on the soil microbiota. An overview of the results from the phytotoxicity and residue studies with Beauveria brongniartii will be given and preliminary data on the interaction of BCAs with indigenous soil microbiota will be presented. Possible conclusions concerning the judgement of risks posed by fungal biocontrol agents will be discussed on the basis of existing guidelines issued by European Commission, EPPO, OECD and US EPA.

Risk characterization of a potential biocontrol agent: receptor identification, risk assessment and management

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One of the main constrains of the use of a microbial biocontrol agents (BCAs) as pesticides is the expensive requirements for registration and the limited size of the potential market (organic). The procedure for registration is the same as that of a chemical pesticides. Particular risk aspects associated to chemicals may be intrinsically absent in naturally occurring microbial BCAs and in any case different aspects should be covered. At any stage of the development of a microbial BCA, from laboratory to filed release, the potential benefit in terms of capacity to control the target pest or disease, has to be weighted against the risk (potential hazard) to the researchers, workers as well as non-target plants or animals. The most relevant difference which requests a case by case approach is the intrinsic capacity to multiply of a microbial BCA which lacks to the chemical. Pest and disease control programmes using microbial BCA need to be backed by a strong documentation on their potential impact of the introduced agents on non-target species and on ecosystems as a whole. Similarly as chemicals a risk potential to non-target species must be considered and attempts are being made to determine the nature of this. The current documentation on which aspects should be considered and relative protocols to tests such side effects are poor and some reports even disputed.
In this work we propose a wide approach to characterise human and environmental risks of employing microorganisms as BCA, by analysing every handling step of the pathway of BCA development and use. For every step, we characterized the BCA's possible ecological receptors (living organisms and environment), we assessed receptor's exposure to the BCA as well as the toxicity of the BCA to the different targets. We reported a wide range of monitoring methods, in order to assess the spatio-temporal environmental fate of the BCAs. The most effective methods are based on molecular biological techniques; very sensitive, with high specificity and relatively simple to apply. We further provide a literature review indicating the microorganism species or genera that constitute hazards to living organisms, in order to classify them in risk classes.

This work wants to deliver an orientation to policy makers developing new European Guidelines for microbial BCA registrations as pesticides and handling procedures guaranteeing safety for people (researchers, technicians, etc.) involved in the R & D projects on new BCAs.

Safety in research: biocontrol agents and semiochemicals risk management in the laboratory

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Occupational safety and health in work environments of research and development of semiochemical and microbial biocontrol agents is currently not regulated by specific standards or directives. In the USA, the Occupational Safety and Health Administration (OSHA) proposes to adopt the standards, which address indoor air quality in indoor work environments. OSHA gives also the rules that justify the use of respirators to prevent the inhalation of harmful airborne contaminants that are alive or were released from a living organism and that cause a range of diseases. In the EU, the European Agency for Safety and Health at Work acts as a catalyst for developing, collecting, analyzing and disseminating information that improves the state of occupational safety and health, but at the moment there are no specific directives on this subject.

Exposure to fungi and molds can cause symptoms such as nasal stuffiness, eye irritation, or wheezing. People with allergies to molds, may have severe reactions. Persons with a reduced immuno-system activity may be at risk to develop mycoses. Therefore isolating and managing unidentified microorganisms could be harmful if precise procedures are not followed. Some episodes of allergies have been reported among people involved in rearing insects for experimental trials.

The aim of this work was to characterize the risk conditions related to dealing with unidentified microorganisms and to rearing insects in the lab, by analysing and replanning of job processes. In particular we dealt with the procedures required in research and development of BCAs and semiochemicals. In this study the method of "congruenze organizzative" developed by Maggi has been used. The resulting document was used as guideline during projecting a new building hosting BCA-research.

The risk related to manipulating unidentified microorganisms, mass production of potential BCAs and rearing insects was characterized. Potential risky situations (separately classifying risks and harms) were identified. Using a draft project of the building, the potential risk situations were analyzed following the precise pathway, related to each procedure that can be harmful for workers. Risk of accident, risk related to manipulation chemicals and unknown substances or biological agents were identified. Identified risk situations were eliminated through specific modification in the preliminary project or by proposing alternative pathways and novel solutions.. Precise protocols were formulated for each procedure. Advices for individual protection were included whenever necessary. Guidelines for planning and managing a laboratory that deals with research and development of BCAs and semiochemicals were produced. These guidelines can also be a valuable tool for increasing safety in labs which already working in this field of research.

Oral Session 3: Management of soil borne diseases

Eradication of plant pathogens and pests from composting wastes and their use in disease suppression

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Currently, landfill is the most popular route for disposal of vegetable wastes in Europe. With the decrease in the availability of landfill sites, and the need to reduce the quantity of biodegradable waste disposed in this way in accordance with the EU Landfill Directive, an alternative to landfill disposal is required. One possible option is to compost the waste, to eradicate any pests and pathogens present, and then return the composted waste to land. Soil incorporation of composted wastes is known to improve various soil properties. In addition, composted wastes have been shown to suppress various soil-borne pathogens and hence disposal of wastes in this way offers the possibility of sustainable disease control. The objectives of this work were to determine the conditions required to eradicate a number of organisms from composting waste and the potential of different composts to suppress soil-borne diseases.

Small-scale flask experiments were conducted to determine the effect of composting conditions on various plant pathogens and pest species. The organisms (*Fusarium* oxysporum, *Pythium ultimum, Olpidium brassicae, Rhizoctonia solani, Plasmodiophora brassicae, Xanthomonas campestris*, Tobacco mosaic virus, *Delia antiqua* and *Psila rosae*) were placed in the centre of horticultural wastes in flasks and incubated in thermostatically controlled water baths for up to 7 d at temperatures ranging from 30-90^{o}C. Following incubation, the viability of the organisms was determined. Most of the organisms examined were either completely destroyed or their populations significantly reduced above 55^{o}C for 7 days. A temperature of 80^{o}C was required to destroy the Tobamovirus, Tobacco mosaic virus. Glasshouse pot bioassays were used to determine the effect of compost incorporation on suppression of root rot (*Phytophthora nicotianae* and *R. solani*) on tomatoes and club root (*P. brassicae)* on Chinese cabbage. The incidence of both the root rots (*R. solani* and *P. nicotianae*) and club root was significantly reduced in the presence of the composted wastes. The level of suppression varied with the inoculum dose and type and rate of compost incorporation.

The use of *Metarhizium anisopliae* against grape phylloxera: first results

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Grape phylloxera, *Daktulosphaira vitifoliae*, is a serious pest of commercial grapevines worldwide. On fresh roots of vines, the grape phylloxera causes so called nodosities, beak-like swellings, as a result of feeding activity. High populations of this pest can result in premature defoliation, reduced shoot growth, reduced yield, and reduced quality of the crop, and even crop death. Currently, registered chemical or biological control agents against phylloxera are not available in Europe. The use of systemic insecticides (e.g. Thiamethoxam and Imidacloprid, respectively) is recommended in California (UC Pest Management Guidelines 2003) but it is very restricted, e.g. to irrigative farming systems and very short entry levels. As a real alternative BCAs should be considered.

In a bioassay, where cleaned nodosities with visible phylloxera occupation were placed on moist filter paper, we tested the principal susceptibility against *M. anisopliae* by spraying with a spore suspension. First infected insects could be found 5 days after treatment. In pot experiments phylloxera-infected grapes were inoculated with barley colonised with *M. anisopliae*. Thirty-two days after inoculation, only old nodosities without any living phylloxera could be found in eight of ten treated plants. On two plants fresh nodosities with few living insects and eggs could be observed only. The control group was still heavily infested with grape phylloxera of all instars. A field was study initiated in 2003. The efficacy of treatment with *M. anisopliae* colonised barley against grape phylloxera was evaluated. The decrease of phylloxera infestations corresponds with the *M. anisopliae* density in the soil. Based on this study, future work will be required to verify the efficacy of new and commercially available M. anisopliae products against grape phylloxera.

Multi-target Biocontrol Efficacy of *Clonostachys rosea* IK726

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Clonostachys rosea, IK726 was originally selected as an effective biocontrol agent (BCA) against cereal seed borne diseases caused by *Fusarium culmorum* and *Bipolaris sorokiniana*. However, the antagonist also controls *Alternaria radicina* and *A. dauci* on carrot seeds and *Ciboria* in acorns. Also diseases caused by soil borne *Pythium* spp. in agricultural and horticultural crops, such as cabbage, carrot and sugar beet, can be reduced. *Pythium* control in forest nurseries with Nordmann fir is currently investigated. Biocontrol in natural environments has been achieved at temperatures ranging from 6º to about 20ºC by dried and stored formulations of the BCA, which strengthens the commercial potential of the strain. *C. rosea,* IK726 can thrive and survive in very different niches. Conidial germination, colonization and conidiogenesis have been shown in natural soil, in vermiculite and on carrot and barley seed and roots as well as on barley leaves. Following seed application, IK726 reproduces and survives several months in the rhizosphere of barley and carrot from natural soils. The development of a gfp-transformant of IK726 that resembles the wildtype strain in ecological fitness parameters and the UP-PCR technology has been very helpful tools in these studies.

Besides traditional ways of applying the BCA, we investigate the feasibility of integrating *C. rosea* IK726 into different physiological seed treatment methods that are characterized by high seed moisture contents. (1) The positive effect on seedling establishment by hydro-priming of carrot seeds can be counteracted if the seed lot is infected by fungal pathogens. However, use of biopriming where IK726 is included in the priming process results in good establishment of IK726 on the seed coat and almost eradication of the indigenous fungal microflora. (2) Recalcitrant seeds must be stored at high moisture content and at low temperature that favours growth of pathogenic and saprophytic fungi. IK726 established on the acorns during several month of storage, which positively affects survival of the acorns. In summary *C. rosea* IK726 has proved to be effective against seed-and soil borne diseases in different crops.

Analysis of efficacy of a biocontrol agent to reduce the transmission of infection in damping-off epidemics

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For many soil-borne pathogens, the reservoir of inoculum in soil is a critical component of the dynamics of an epidemic, with the source of inoculum being able to persist for long periods in the soil. There are therefore two processes driving soil-borne epidemics: primary infection from resident (or incoming) inoculum, followed by newly infected host tissue from transmission between diseased and healthy plants. Widely used empirical models for disease progress (such as mono-molecular or logistic) cannot summarise these dynamics adequately. Nevertheless, these models are typically used to analyse the efficacy of control strategies on epidemics. Whilst this allows for an empirical comparison, it tells us little about how biocontrol reduces the transmission of infection from diseased to healthy plants in a population. In this paper we present a simple analysis based on generic epidemiological processes to quantify the efficacy the fungus *Trichoderma harzianum* to control damping-off disease caused by *Rhizoctonia solani*.

Damping-off epidemics were studied in replicated microcosms comprising 160 radish plants grown in either sand or soil. Epidemics were initiated by challenging 15 randomly selected plants with *R. solani*. The biocontrol agent Trianum-P (Koppert), which contains the antagonistic fungus *T. harzianum* was added to provide an initial spore density of $4\ 10^7$ spores/g. The time at which each plant became diseased was recorded for a period of 18 days to produce spatio-temporal maps of disease progress. We combine these spatio-temporal maps of disease progress with a generic *S-I* (susceptible-infected) model frequently used to describe plant epidemics, to obtain empirical estimates of the rate of disease transmission between infected and healthy plants. The basic idea behind the analysis is that epidemics are fuelled by *(i)* the number of contacts between infected (*I*) and susceptible (*S*) neighboring plants, and (*ii*) the probability of successful transmission of disease from an infected to a susceptible plant. This enables a simple estimation of the rate of transmission at each time during the epidemic. We define *the efficacy of biocontrol* as the reduction in the rate of transmission of disease, and show that for our epidemics: *i*) the efficacy of biocontrol was different for primary and secondary infection; *ii*) the efficacy of biocontrol was affected by the soil medium, and *iii)* the efficacy of the control agent was not constant throughout the epidemic as it failed to control disease during the later stages of an epidemic. The potential of this type of analysis to an epidemiological understanding of the efficacy of biocontrol agents is discussed.

Recent developments in inoculum production, application and pathogenicity in the biocontrol agent *Coniothyrium minitans*

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Coniothyrium minitans is a sclerotial mycoparasite of the plant pathogen Sclerotinia sclerotiorum and is used commercially as a disease biocontrol agent. However, knowledge of inoculum production and application for optimal efficacy is limited and little has been done to characterise the genes and gene products involved in this mycoparasitic interaction. Consequently, over the last few years, a collaborative series of studies examining these key features associated with biocontrol have been carried out. Both packed bed reactor and liquid culture systems were used to produce conidia of C. minitans and sclerotial-based parasitism assays were devised to assess inoculum quality. Spray drying of conidia from packed bed reactors containing oats or nutrient impregnated hemp maintained viability and infectivity following storage for 6 months at 5^{o}C, irrespective of whether the conidia were obtained from parts of the reactor exposed to high temperatures (above 30^{o}C), or from areas maintained at lower temperatures, optimal for mycelial growth. In liquid culture in potato dextrose broth, production of high levels of conidia (10^7/ml) was dependent on the C. minitans isolate used and reflected ability to produce mycelial strands rather than pellets in shaken culture. Conidia produced in liquid culture were equally effective at infecting sclerotia as those produced on potato dextrose agar (PDA). These studies indicate that using appropriate nutrient sources and isolates, successful production of effective C. minitans conidia can be achieved in both solid state and liquid fermentation systems. When conidia from PDA were applied at 10^6 cfu/g soil to three soil types which had been sterilised, pasteurised or left non-sterile and then stored at 18^{o}C for 30 d, there was no decrease in cfu throughout the experiment. Proliferation was observed when lower inoculum rates were applied in sterilised soil but not in pasteurised or nonsterile soil. Use of a hygromycin resistant strain enabled detection of C. minitans in four soils and from decaying sclerotia after 8 weeks incubation at 20^{o}C. This work demonstrates the ability of C. minitans to survive in a range of soils and in sclerotia for considerable periods of time. To identify novel pathogenicity genes in C. minitans, restriction enzyme mediated integration (REMI) transformations were carried out and eight putative nonpathogenic mutants were identified from a collection of over 3500 transformants. Plasmid rescue and sequencing of the flanking regions have commenced and preliminary BLAST analyses indicate that genes other than those coding for chitinases and glucanases may be involved in pathogenicity. Interestingly, antifungal antibiotics have recently been identified from some isolates of C. minitans and their role in pathogenicity is also under investigation.

TUSAL®, a commercial biocontrol formulation based on *Trichoderma*

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Trichoderma is a natural and cosmopolite fungus that offers an environmentally friendly approach to the management of plant diseases. It can be incorporated into cultural and physical control methods and integrated with limited chemical usage for effective IPM strategies. *Trichoderma* is a useful biocontrol agent of plant pathogens suitable for protecting or treating plants and plant material against infections and diseases caused by phytopathogenic organisms; to stimulate plant growth; to enhance root system; to induce systemic resistance in plants and to control biodeterioration agents of materials. There are several *Trichoderma* species recognized as biocontrol agents and their biodiversity opens an array of potential combinations since different strains can be mixed in a single formulation. Using this strategy, we have combined different strains of *Trichoderma* in both liquid (osmotically regulated) and solid (wettable powder or granules) formulations, not only for soil or plant applications but also for coated seeds. The progress in formulation designs, including production efficacies and field results, is shown in pathosystems such as sugar beet damping-off, sugar beet *Rhizoctonia* root rot, sugar beet *Phoma* root rot, rhizomania virus disease (control of the fungal vector *Polymyxa betae*), lettuce drop (*Sclerotinia sclerotiorum* and *S. minor*), strawberry diseases (*Colletotrichum*, *Rhizoctonia* and *Phytophthora*) and pepper soil diseases. At present, one of the formulations based on a cocktail of strains of *T. harzianum* and *T. viride* (TUSAL®) is being registered as fungicide in Spain and the EU. However, why the ratio between registered *Trichoderma* fungicides vs *Trichoderma* fertilizers is so low? This question has many answers.

Oral Session 4a: Mode of action of biocontrol agents

Endophytes: A new source for multi-target Biological Control Agents?

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Endophytes are an interesting group of plant-associated bacteria that live inside plants and show neutral or beneficial interaction with the host plants. The structure of the bacterial community in the endosphere and endorhiza of field-grown potato was analysed in comparison to the rhizosphere and phyllosphere by a multiphasic approach over a period of three years. Results of the cultivation-independent approach revealed that T-RFLP profiles of the two endophytic microenvironments were more closely related than those from ectophytic habitats. Using cultivation, composition, diversity and richness of bacterial antagonists confirmed specificity for each microenvironment and an outstanding and promising role of the endorhiza. In an approach to measure the biocontrol potential of isolates, a total of 2,648 bacteria evaluated for biocontrol and plant growth promotion by a hierarchical combination of assays using the soilborne plant pathogens *Verticillium dahliae* and *Rhizoctonia solani* as target. An average of 14.4% of examined bacteria expressed antifungal properties. The strains were characterized by their antagonistic mechanisms *in vitro* as well as their production of the plant growth hormone indole-3-acetic acid. Complementary, the plant growth promoting effect by antagonistic bacteria was determined using a newly developed microplate assay on the basis of lettuce seedlings. Seven endophytic isolates selected according to *in vitro* criteria were evaluated in greenhouse and field trials regarding their efficiency to control *Rhizoctonia solani* in lettuce, sugar beet and potatoes. In addition, they were screened for their biocontrol activity against the pathogenic nematode *Meloidogyne incognita.* The most promising candidates *Pseudomonas reactans* 3Re2-7 and *Serratia plymuthica* 3Re4-18 will be commercialised as biological control agents.

Novel understanding of the biocontrol mechanisms of *Trichoderma*, a mycoparasite and an opportunistic avirulent plant symbiont

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Trichoderma-based biofungicide are a reality in commercial agriculture, with more than 50 formulations registered worldwide as biopesticides or biofertilizers. Several research strategies have been applied to identify the main genes and compounds involved in the complex, three-way interactions between fungal antagonists, plants and microbial pathogens. Proteome and genome analyses have greatly enhanced our ability to conduct targeted and genome-based functional studies. We have obtained reproducible 2-D maps of the entire fungal proteome in various conditions of interaction, which permitted the isolation of many proteins related to specific functions. Many differential proteins from several biocontrol strains of *Trichoderma* spp. during the *in vivo* interaction with different plants and/or several phytopathogenic fungi have been isolated and analysed by MALDI-TOF. Relevant genes have been cloned and specifically inactivated, to demonstrate their function in biocontrol and induction of disease resistance. GFP-based reporter systems with interaction-inducible promoters allowed the characterization of regulatory sequences activated by the presence of the pathogen or the plant. From extensive cDNA and EST libraries of genes expressed during *Trichoderma*-pathogen-plant interactions, we are identified and determined the role of a variety of novel genes and gene-products, including ABC transporters specifically induced during antagonism with other microbes; enzymes and other proteins that produce or act as novel elicitors of Induced Resistance in plant and promote root growth and crop yield; proteins possibly responsible of a gene-for-gene avirulent interaction between *Trichoderma* and plants; mycoparasitism-related inducers released from fungal pathogens and that activate biocontrol in *Trichoderma;* fungal promoters specifically induced during mycoparasitism and plant colonization; plant proteins and a novel phytoalexin induced by the presence of the fungal antagonist; etc. We have also transgenically demonstrated the ability of *Trichoderma* to transfer heterologous proteins into plants during root colonization. Finally we have used GFP and other markers to monitor the interaction *in vivo* and *in situ* between *Trichoderma* and its host(s) (the fungal pathogen and the plant).

Studies on induced resistance against fire blight (*Erwinia amylovora*) with different bioagents

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Three different bioagents (BION®, etheric oil from *Thymbra spicata* and the antagonistic bacterium *Rahnella aquatilis* Ra39) were tested on their efficacy against fire blight (*Erwinia amylovora)* and on their resistance induction activity. The experiments were carried out under controlled climatic conditions in the greenhouse. For the studies M26 apple rootstocks were used as host plant. Moreover as a marker of resistance in physiological studies the total phenol content and enzymatic activity of polyphenol oxidase (PPO) were estimated.

The treatments with BION®, etheric oil and Ra39, resulted in a reduction of the disease index of up to 63.7, 30.8 and 58.6% respectively. This was correlated with a decreasing effect on the growth of bacteria up to 64.2, 49.5 % and 63.8% respectively during the course of infection. In physiological studies on apple rootstock shoots significant changes in the total phenol content and activity of PPO were found after BION®, etheric oil and Ra39 treatment. In uninoculated shoots phenol content increased by 103, 75 and 62,5% after 6 days after application and PPO increased by all treatments 203, 206 and 233%. Moreover in inoculated shoots phenol content increased by all treatments 100, 47.9 and 30.4% after 6 days application respectively and PPO activity 73, 28 and 39.7% at 4 days post infection, respectively. Summarizing, the effect of the three bioagents, it can be concluded that they all could cause an induced resistance in the apple rootstocks against fire blight.

Oral Session 4b: Postharvest

Comparative study of the role of chitinase and pyrrolnitrin for biocontrol activity of *Serratia plymuthica* IC1270

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Serratia plymuthica strain IC1270 isolated from rhizosphere of grape is a wide range antagonists of plant pathogens. Under greenhouse conditions, application of the bacterium reduced the incidence of several soil-borne fungal diseases, including *Rhizoctonia solani* root-rot in cotton and bean, and *Pythium aphanidermatum* damping-off in cucumber, by up to 90%. The high potential of strain IC1270 was also shown against *Sclerotium rolfsii* in bean and *Fusarium oxysporum* f. sp. *melonis* in melon. Besides its capability to control soil-borne diseases, strain IC1270 protects plants against *Botrytis cinerea* and *Sclerotinia sclerotiorum* foliage diseases in sunflower, rape and cucumber seedlings. The biocontrol potential of strain IC1270 was also demonstrated against post-harvest diseases on peaches and oranges infected by *Monilinia fructicola*, *Rhizopus stolonifer* and by *Penicillium digitatum*. Strain IC1270 produces several antifungal compounds, including antibiotic pyrrolnitrin (Prn) and chitinolytic enzymes. To clarify the relative input of these compounds in the bacterium biocontrol activity two mutants were obtained by gene-replacement technique. One of the mutants, IC1270#C7, deficient in chitinolytic activity and another one, IC1270#P1, deficient in Prn production, were compared against *R. solani and P. aphanidermatum in vitro* and in greenhouse. Whereas mutant C7 hardly differed from the parental strain, P1 mutant lost its antifungal activity almost completely.

The results demonstrate prevalent role of Prn for strain IC1270 antifungal activity. However, combination of chitinases with Prn that has a direct effect on membranes could be considered an advantage as an aid to the chitinases in their attack on fungal phytopathogens. Other mechanisms, including competition for nutrients and induced resistance, are shown to be involved in strain IC1270 biocontrol capacity as well.

Formulation and shelf-life studies of the biocontrol yeast *Pichia anomala*: positive effect of endogenous solutes, isotonic solutions and additives during fluidised-bed drying

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The major hurdle in production of commercial biocontrol agents (BCAs) has been the lack of production of formulations of the right quality and shelf-life. Of particular importance is the conservation of viability and ecological competence after application. With this in mind studies were conducted to develop production and formulations of *P. anomala* which would have these attributes. *P. anomala* cells were optimally produced in a liquid formulation based on molasses. By manipulation of the physiology of the cells during growth it was possible to modify the endogenous trehalose and arabitol and glycerol content of the cells. Studies demonstrated that by measurement of the internal water potential of the cells it was possible to suspend in isotonic solutions based on NaCl, a solute with no adverse effect on yeast cells viability. Fluidised bed drying conditions of yeast cells were optimised, in terms of drying temperature and time, and cell viability was about 70% with a moisture content of 7%. Subsequent studies examined carriers and adjuvents for conservation of viability after fluidised bed drying. These studies showed that the addition of cotton seed flour+skimmed milk powder or corn starch+skimmed milk powder were best treatments. During a two month-storage period at 4^{o}C, formulated yeast cells with a high trehalose intracellular concentration, retained high viability (up to 70%). Moreover, use of isotonic solutions resulted in improved shelf-life of formulated yeast cells; cells suspended in water were 55% viable while those suspended in NaCl isotonic solutions retained 70% viability for the same storage period. These treatments were effective at controlling *Penicillium* and other spoilage fungi in moist grain.

Postharvest biological control of a wide range of pathogens and fruit types by *Pantoea agglomerans* EPS125

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Pantoea agglomerans EPS125 was isolated using a selective enrichment technique from a Doyenne du Comice pear. The strain can be specifically identified by means of DNA fingerprinting using MRFLP patterns resolved by Pulsed Field Gel Electrophoresis, can be produced at semi-industrial scale by liquid fermentation and freeze drying and is the object of a patent (WO 02/098233 A1). EPS125 efficiently inhibit infections by Penicillium expansum in apple and pear fruits either in commercial cold storage conditions under controlled atmosphere or in simulated market conditions, Monilinia laxa and Rhizopus stolonifer in stone fruits (plum, peach, cherry, apricot and nectarine) and Botrytis cinerea in strawberry fruits. The efficacy of biocontrol depends on the concentration of the biocontrol agent and pathogen. At medium to low pathogen dose, optimal EPS125 concentrations are above 10^7 CFU ml^{-1}. The strain EPS125 colonizes, grows and survives well on wounds and less in non-wounded peel surface of pome and stone fruits. Significant inhibition of conidial germination and hyphal growth of P. expansum, R. stolonifer and M. laxa is achieved when the fungal and EPS125 cells are co-cultivated on peel leachate, nectarine juice or apple juice. However, no effect is observed when the antagonist and the pathogen cells are physically separated by a membrane filter, which permits nutrient and metabolite interchange. Therefore, a direct interaction between the strain and the pathogen cells is necessary for antagonism, without a significant contribution of the production of antibiotic substances or nutrient competition. Preemptive exclusion by wound colonization and direct interaction with the pathogen is proposed as the mechanism of biocontrol. Transposon mutagenesis is being performed to demonstrate the mechanisms of biocontrol. A total of 4000 mutants have been obtained with minitransposon GUS and are currently characterized and tested by in vitro and ex-vivo assays.

Oral Session 5a: Combination of control means

Integration of the use of the antagonist *Ulocladium atrum* in management of strawberry grey mould (*Botrytis cinerea*)

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Botrytis cinerea is causing fruit rot of strawberry after infection of flowers and fruits by airborne conidia. Conidia of *B. cinerea* can be produced on necrotic tissues such as necrotic leaves of the strawberry crop but also on various other necrotic plant tissues inside and outside the crop. The antagonist *Ulocladium atrum* has been selected for its ability to compete with *Botrytis* spp. in necrotic tissues under a broad range of environmental conditions. Antagonism results in suppression of sporulation of the pathogen, as shown in field experiments in onions, and in restricting growth of *Botrytis* from necrotic plant tissue to adjacent healthy tissue, as demonstrated in cyclamen under commercial conditions.

Preliminary experiments in strawberry demonstrated the potential of *U. atrum* to suppress *B. cinerea* sporulation on strawberry leaves as well as to protect flowers from invasion by the pathogen. The antagonist did not affect pollination and fruit formation. Based on these results, a series of ten field experiments has been carried out between 1996 and 2003 to optimise and to integrate the use of *U. atrum* in control of grey mould in annual strawberry crops. First experiments were conducted to identify the relevant targets for the antagonist in the crop. Necrotic strawberry leaves are no significant inoculum source of *B. cinerea* in annual strawberry crops. Consequently, application of *U. atrum* before flowering on the transplants or on the crop did not result in disease control. In the same experiments, applications of *U. atrum* or fungicides during flowering reduced grey mould incidence significantly by 33 % and 57 %, respectively. The results of subsequent field experiments demonstrated that intervals between antagonist applications during flowering must be short to guarantee that newly opened flowers are reached by the antagonist. When a regular application regime was applied, at least two applications per week were found to be necessary. In 2002 and 2003, field experiments were carried out using a decision support system (DSS) to optimise timing of antagonist applications and to avoid applications under conditions not favourable for disease development. In 2002, eight applications of *U. atrum* at three to four-day intervals resulted in a reduction of grey mould incidence by 50 %. When applications were timed according to DSS (at an infection chance >7.5 %), four applications were carried out resulting in a reduction of grey mould incidence by 30 %. In 2003, seven applications of *U. atrum* at three to four-day intervals resulted in a reduction of grey mould incidence by 24 %; six applications timed according to DSS resulted in a reduction of grey mould incidence by 39 %. Fungicide application timed following the same decision rules reduced grey mould incidence by 67 %.

Next research steps will be the integration of the use of fungicides and antagonist in a control strategy. Disease control may be improved when the antagonist is applied at periods of moderate infection risks, but fungicides at periods of high infection risks. Integration of powdery mildew control has also to be considered. When the disease occurred in field experiments, fungicides aimed at grey mould control but not the antagonist achieved powdery mildew control.

Suppression of *Rhizoctonia* root rot of tomato by *Glomus mosseae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with combined modes of action

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Root-rot due to *Rhizoctonia solani* is a major soilborne disease causing growth and yield depression. The ability of *Glomus mosseae* BEG12 and of *Pseudomonas fluorescens* A6RI to suppress this soilborne disease in tomato was assessed by comparing the shoot and root growth of infested plants when protected or not by these beneficial strains. Disease suppression of soilborne diseases is commonly ascribed to both reduction of the epiphytic (microbial antagonism) and parasitic (induced resistance) growth of the pathogen. The pathogen growth at the root surface and in the root tissues was then assessed by microscopic observations. The root architecture of the tomato plants in the different experimental conditions has been further characterized by measuring total root length, mean root diameter, number of root tips and by calculating root branching degree. Data obtained indicate that both beneficial strains suppressed root-rot due to *R. solani*. This suppression was related for both organisms to microbial antagonism and induced resistance. On the top of these modes of actions, the increase of the root length and number of root tips appeared to also contribute to the disease suppression.

Biological control of *Pythium aphanidermatum* in cucumber with combined applications of bacterial antagonists with chitosan

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Pythium aphanidermatum (Edson) Fitzp. is an important disease in greenhouse vegetables grown on soilless substrate systems, especially if the nutrient solution is recirculated. The disease is difficult to control. In the short term, there are no prospects for breeding resistant cultivars, and fungicides (i.e., propamocarb and metalaxyl) are only effective if used as preventive applications. Used rockwool slabs can have disease suppressive properties due to the autochthonous microflora, but this is not always a suitable solution due to the risk of other diseases. Many years of research on biological control agents has resulted in several fungal and bacterial isolates with biocontrol properties, but their effect is often insufficient or not reproducible. With the purpose to obtain more reliable biocontrol agents, many bacteria have been collected from suppressive rockwool. Their antagonistic properties were tested in small bioassays. The most effective organisms occurred to be *Lysobacter enzymogenes* isolate 3.1T8 originating from young roots, and a filamentous actinomycetes *Streptomyces* sp. isolate B65 originating from old roots in used rockwool slabs. Nevertheless, larger scale experiments with these isolates in an ebb and flow systems with recirculated nutrient solution were disappointing. Therefore, combinations of the two bacteria with chitosan, which is known as an elicitor, were tested. The combination of two bacteria alone did not give any biocontrol effect. But all combinations between the two bacteria and chitosan were highly effective; the disease suppression was 60-95 % as compared to the control with only *P. aphanidermatum*. This synergistic effect between the antagonistic bacteria and a natural product opens new possibilities for the development of a more reliable biocontrol strategy for the control of *P. aphanidermatum*.

Evaluation of the sustainability of strategies that include biocontrol agents to reduce chemical residues on strawberry fruits

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Biocontrol agents (BCAs) have become one of the most promising tools to be developed for reducing the use of chemical pesticides both against insects and diseases. Despite of some important benefits (reduced pesticides residues, safety for operators, general benefits for environment), the use of BCAs has some drawbacks if compare with chemicals which prevent BCAs large commercial diffusion. The economic aspect is probably one of the most important factors that has to be considered in developing a BCA. Measuring the potential success of a BCA based strategies to protect plants against pest and pathogens must be important in particular in the first stage of the research and development of it. Several are the BCAs that even if active against a pathogen have not found a commercial future in field application. It is increasingly agreed among researchers that we need better common understanding of the impacts of implementing BCA.

Economic evaluation tools have been used only to a little extent. Moreover, in a sustainability approach attention has to be paid to environmental and social aspects.

This study focuses on the development of a sustainability framework for the selection of the best combination of BCAs in integrated strategies to reduce chemical input, after evaluating all the important factors, including economical, social and environmental ones.

This study is based on the data obtained from an integrated pest management experiment of strawberry in the Trentino Region in which BCAs were used to reduce the chemical input. Strawberries are particularly interesting because "they are world-wide produced with practices that many consider unsustainable" and have been included in the "list of top-ten foods to avoid because of high pesticide residues". Chemical based strategies are compared with strategies based solely on BCA or BCAs integrated with chemicals. The different techniques are described and costs and yields from two years open-field testing are compared with average costs and yields from comparable farms in the same area. Data on pesticides residues are also introduced in the assessment framework. Marketing organisation, market conditions and social acceptability are discussed in order to highlight necessary conditions for a successful biological control from a sustainability point of view.

Oral Session 5b: Integrated management of diseases

Use of biocontrol agents against powdery mildew in integrated strategies for reducing pesticide residues on strawberry: efficacy and side effects evaluation

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Pesticide residues became a major issue that resulted in legislative actions that limit and regulate pesticide use. The upper legal limits of a pesticide residue to be found on a food (maximum residue level, MRL) have been fixed in all countries. The MRL is not a toxicological limit and its violation is not necessarily a cause of concern for public health. In fact for authorized pesticides, the MRLs are set at the maximum safe level that one would expect if the pesticide is used according to the rules and restrictions specified in the authorisation. However, consumers and policy makers are demanding lower pesticide input to increase quality and safety of food along the whole food chain, without increasing costs to farmers and consumers. Strawberry powdery mildew (*Sphaerotheca macularis* f.sp. *fragariae*) is a serious disease of strawberry in warm and dry climates or in greenhouses. Off-soil productions in greenhouses have several positive effects on fruit quality and post-harvest keeping and they allow expanding the production period. They also help in the control of several important diseases like grey mould, anthracnose or root rots. However, without the inhibitory effect of rain on conidia, they result in an increase of powdery mildew incidence. *S. macularis* is controlled primarily by chemical treatments. A at least six-seven treatments are required for each growing cycle in off-soil plantation in tunnels. The purpose of this four years work was to evaluate the efficacy of alternatives to chemical pesticides and their integration into strategies against powdery mildew of strawberry with the aim of reducing pesticide residues on fruits. The influence of the proposed strategies on the mite pest tetranychid and predatory mites was also evaluated. The most widely used fungicides against powdery mildew on strawberry were compared and several alternatives (salts, plant extracts and biocontrol agents) were tested for efficacy in controlling *S. macularis* in greenhouse and experimental field trials. The best strategies of chemical-non chemicals-combinations were tested for two years in two sites. Salts (bicarbonates, carbonates) that seemed to have an effect on the disease were scarcely active under field conditions. Biocontrol agents (BCAs), like *Ampelomyces quisqualis*, *Bacillus subtilis* and *Trichoderma harzianum* T39, can control the disease, but to a lesser extent as compared chemicals, and their level of activity varies among location, environment and time of application. When BCAs were applied in combinations with chemicals a good reduction of residues was achieved while maintaining the same control efficacy against. All the proposed strategies did not increase *Tetranychus urticae*, they had no side effects on *Amblyseius andersoni* populations and consequently they can be regarded as safe for the ecological balance. A further reduction of pesticide application and the optimization of the integrated strategies will still be possible with a better understanding of the epidemiology of the disease and the identification of the optimal conditions for BCAs applications in field.

Integrated management of late blight in greenhouse tomatoes

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Late blight, caused by *Phytophthora infestans*, is one of the most devastating diseases of greenhouse grown tomatoes in Israel and elsewhere. For its suppression, growers frequently apply fungicides, but it is not uncommon that severe epidemics develop even in fungicide-treated crops. Determining the quantitative effects of the relevant factors on the pathogen may lead not only to better disease suppression, but also to reduction in fungicide use. The effects of various management actions (covering the soil with plastic, application of fungicides and sanitation), and their interactions, were studied in a series of experiments conducted in walk-in tunnels and commercial-like polyethylene greenhouses. Under conditions of the western Negev (south west Israel) it was found that foliar infection by the pathogen could be suppressed by covering the soil with reflective polyethylene (that resulted in reduction of relative humidity and leaf wetness duration) and by application of fungicides. Under conditions of high temperatures (>20^{o}C) and dry foliage the infections do not occur. Nevertheless, the pathogen progresses from infected leaf-blades via the petioles, to the stems, where it causes stem lesions. Stem lesions eventually lead to plant death. Observations made in the greenhouses suggested that the damage resulting from stem infection is more significant than that resulted from foliar infection. It is possible to prevent stem infections by sanitation, i.e. removal of infected plant material. Moreover, it was observed that the rate of disease progression in infected leaves was reduced, and fewer plants died from stem lesions, when temperatures exceeded 30^{o}C. Accordingly, avoidance from opening the side-opening of the greenhouse during the day (which resulted in increased daily temperature) enabled to further improve disease management. In conclusion, the combination of cultural means and spraying control agents at various stages during the growth season results in significant reduction of tomato late blight in greenhouses.

An integrated approach to simultaneously control insect pests, powdery mildew and seed borne fungal diseases in barley by bacterial seed treatment

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In connection with extensive testing of a large number of bacteria for biological control of fungal seed borne pathogens, we have noted on several occasions that application of these bacteria to seed also protects against insect pests and diseases attacking aerial plant parts. Thus a single treatment can offer the plants protection against a large variety of agents. The method of applying bacteria to seeds of cereals has been developed for large scale commercial use by BioAgri AB (Uppsala, Sweden) in the product Cedomon™, which is based on a strain of *Pseudomonas chlororaphis*. Twenty eight diverse bacterial strains representing diverse taxa isolated from different Swedish and Moroccan soils were tested for induction of resistance to powdery mildew in barley (*Blumeria graminis* f. sp. *hordei*). Seeds coated with bacterial culture broth resulted in plants that either were more susceptible or resistant to powdery mildew. The four strains that induced the highest level of resistance to powdery mildew were tested more stringently in repeated tests. They were also tested for protecting plants from leafhoppers (*Psammotettix alienus*). A pot containing plants that had emerged from untreated seeds was placed in the same insect cage as one that contained plants that had emerged from treated seeds. The pots had no physical contact, even through irrigation water. Several leafhoppers were placed in each cage when the plants were 10 days old. The feeding preference of the leafhoppers toward plants emerging from treated vs. those that emerged from untreated seeds was recorded over a 14 day period. The results from repeated glasshouse trials indicate that certain strains of bacteria have a great potential to control not only seed borne diseases of cereals, but also to induce resistance to leafhoppers and powdery mildew, whereas others only induce resistance to leafhoppers. The diversity in the response of plants to bacterial seed treatment is intriguing. Upcoming field trials will study the persistence of this resistance response under field conditions. The most effective strains will be candidates for commercial development of multipurpose biocontrol agents.

Development of a management system for integrated and biological control of *Botrytis* spp. in flower bulb crops

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Botrytis elliptica and *Botrytis tulipae* are the causal agents of leaf blight in respectively lily and tulip (also called 'Fire'). These *Botrytis* spp. are responsible for losses in lily and tulip production up to 80% (dependent on cultivar). To control Botrytis a significant amount of fungicides (25-40 kg/hectare) is used during the growing season. Currently the use of fungicides is under discussion due to environmental concerns and development of fungicide resistance. Moreover, in biological production of lilies and tulips Botrytis leaf blight is one of the big problems. At PPO-Flowerbulbs a management system is developed in which several measures are combined resulting in control of leaf blight with no or as less fungicides as possible. Basis for this management system is a Botrytis warning system. Through forecasting infection periods it reduces the number of fungicide applications and improves spray efficacy and therefore Botrytis control. In such a system other control methods than fungicides can be used also to further reduce fungicide input. Different antagonistic micro-organisms (e.g. yeasts, Pseudomonas bacteria) and plant extracts, essential oils etc. are being tested for their Botrytis controlling abilities and some have good potential. Furthermore early detection of Botrytis in the field may improve the warning system. In addition, other ways to reduce Botrytis infection like less dense planting, N-fertilisation (under investigation), crop residue management will be implied into the management system in order to achieve good Botrytis control with no or less fungicides.

Poster Session 1

The binary pesticide activity of *Bacillus thuringiensis*

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Plant protection includes control of harmful insects and plant diseases. At present there are no described bacteria-based formulations with binary (insecticidal and fungicidal) activity. Some of the work conducted at our laboratory is oriented towards finding such bacteria. From the large collection of over 300 strains with biocontrol potential we have singled out several strains *Bacillus thuringiensis*, which produce protein crystals and inhibit fungal growth. These strains inhibit the growth and germination of phytopathogenic fungi *Fusarium graminearum*, *Fusarium solani*, *Fusarium nivale*, *Sclerotinia sclerotiorum, Rhizoctonia solani.* The level of synthesis of fungicidal factors is dependent on the cultivation media used. The fungicidal activity of the strains is localized in supernatant. The fungicidal factors of supernatant keep its activity after treatment by proteolytic enzymes and heat. Currently we are testing the activity of these strains using various test insects and investigate chemical structure of the fungicidal factors.

Use of chemical elicitors to reduce insect pest populations in greenhouse tomatoes

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Recent advances in the field of chemical ecology have identified many chemical elicitors that trigger induced resistance and systemic acquired resistance in a variety of plants, including many crop species. Exogenous applications of elicitors have been demonstrated to cause expression of a variety of proteinase inhibitors or elevated levels of certain plant oxidative enzymes that in turn are correlated with reduced pests populations on "induced" plants. We report the results of preliminary experiments to assess the feasibility of using applications of four elicitors, Actigard®, Messenger®, 2-chloroethylphosphonic acid, and methyl jasmonate to reduce populations of insect pests commonly infesting commercially grown greenhouse tomatoes in Pennsylvania. Application of 2-chloroethylphosphonic acid and methyl jasmonate caused significant elevations in the levels of peroxidase and polyphenol oxidase in tomato leaf tissue. In studies of tomato plants infested with green peach aphids, *Myzus persicae*, applications of the elicitors Actigard® and methyl jasmonate significantly depressed the rate of aphid population growth. Further studies are underway to optimise the timing of elicitor applications and to determine if other greenhouse pests or diseases are affected by elicitor applications. Biological control failures are frequently attributed to the inability of insect pathogens and natural enemies to keep up with rapid development and reproduction of the target pest. Slowed pest development and reproduction following application of elicitors to crops, may offer improved opportunities for successful biological control.

Phenotypic traits underlying wound competence of postharvest biocontrol yeasts and degradation of mycotoxins by these microorganisms

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We had previously shown that wound competence of postharvest biocontrol agents (BCAs) acting against wound pathogens of apples relies on resistance of these microorganisms to reactive oxygen species (ROS: superoxide anion, $O_2^{-}\cdot$ and hydrogen peroxide, H_2O_2), generated by the fruit tissue as a consequence of wounding. In this work we report the *in vitro* comparison of the enzyme activities deactivating ROS (superoxide dismutase, SOD and catalase, CAT) and the antioxidant potential of culture filtrates of two biocontrol yeast strains displaying lower (*Rhodothorula glutinis* LS11) and higher (*Cryptococcus laurentii* LS28) wound competence and antagonistic activity. The more efficient antagonist LS28 showed significantly higher SOD and CAT activities and higher antioxidant potential of its culture filtrate. Recently, the EU has released regulations (472/2002 and 1425/2003) setting the highest tolerable levels of the mycotoxins Patulin and Ochratoxin A (OTA), which contaminate apple-based food products and wine, as a consequence of infections by *Penicillium expansum* and *Aspergillus carbonarius* on apples and grape, respectively. Two biocontrol agents of our collection (strain LS11 and *Aureobasidium pullulans* LS30) are able to degrade *in vitro* Patulin and OTA, respectively. Further, strain LS11 shows active reduction of Patulin also *in vivo*, i.e. in apples artificially infected by *P. expansum*.

Multi trophic relationships - interaction of a biocontrol agent and a pathogen with the indigenous micro-flora on bean leaves

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Research on microbial interactions in agricultural ecosystems has yielded a vast amount of knowledge about biocontrol of plant pathogens. Infection by pathogens can be reduced by prior inoculation of the plant surfaces with antagonistic microbial agents. In spite of the potential effect of phyllosphere microflora on the interaction of pathogens and BCAs with the host plants, and on the outcome of biocontrol activity in agricultural systems, this subject has until now received only minor attention. The phyllosphere microflora may interact with the pathogen, the BCA and the plant and affect the outcome of their interaction. The microflora may be altered when an infected plant is treated by a BCA resulting in a change of the outcome of the interaction to give either better control of the pathogen or enhanced disease levels.

We studied bean leaves for multi-level interactions of the biocontrol agent (BCA) *Trichoderma harzianum* T39, the plant pathogen *Botrytis cinerea* and indigenous microflora. *B. cinerea* disease severity on leaves was evaluated following direct *T. harzianum* treatment to the leaves or root treatment. Culturable microflora on leaf surfaces and inside the leaf tissue were studied following either surface washing or surface leaf disinfection and maceration, each followed by conventional plating on growth media. Bacterial 16S ribosomal DNA (rDNA) extraction from bean leaves that were subjected to the various treatments was PCR amplified with the primers 530R and GC-338F. Amplicons were separated by size and base composition by denaturant gradient gel electrophoresis (DGGE) in order to fingerprint shifts in the structure of the natural plant-associated microbial communities (culturable and non-culturable) that may result from biocontrol and pathogen activity.

B. cinerea-incited disease on bean leaves was significantly reduced when leaves were directly treated with *T. harzianum* T39 or when a spatial separation between biocontrol treatment and pathogen leaf infection was kept, i.e. the *T. harzianum* was applied to the root zone and the pathogen was infected on the leaves. Leaf tissue associated, culturable yeast and bacterial population levels were increased as a result of *B. cinerea* infection and as a of *T. harzianum* treatments either to leaf or to roots. Surface phylloplane yeast levels tended to decrease as a result of *T. harzianum* treatments. Representative isolates from the phyllosphere antagonized *Botrytis in vitro* and on bean leaves. DGGE performed with the same samples revealed increased variation in the bacterial population during the course of plant incubation as a result of *B. cinerea* infection. *T. harzianum* treatment to the leaves or to the root zone resulted in increased variability in the bacterial population inhabiting the leaves.

It may be speculated that some of the effect exerted by *T. harzianum* as a biocontrol agent is associated with microbial changes that occur as a result of is direct application to the leaves or indirect application to the roots. Earlier research revealed other modes of action of *T. harzianum* i.e. induced resistance, competition for nutrients and space and suppression of *B. cinerea* pathogenicity enzymes.

Systemic resistance in *Arabidopsis thaliana* induced by biocontrol agent *Trichoderma harzianum*

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The potential of the biocontrol agent *Trichoderma harzianum* T39 (as Trichodex) to trigger induced systemic resistance (ISR) responses was studied in *Arabidopsis*. The response to the foliar pathogen *Botrytis cinerea* and to *T. harzianum* of wild types (WT) and mutants of *Arabidopsis* was tested in forty-three lines and mutants. In ecotype Colombia-0, relatively resistant to *B. cinerea*, *Trichoderma* application at sites spatially separated (root application) from the *B. cinerea* inoculation (leaves) resulted in 25% (up to 70%) reduction of grey mould symptoms. Ecotypes Wassilewskija-4 (Ws-4), Nossen-0 (No-0) and Landsberg-0 (Ler-0) had low level of basal resistance to *B. cinerea*; Ws-4, No-0 and possibly Ler-0 were found to be blocked in their ability to express ISR. Abscisic acid (ABA)-related mutants, both deficient and insensitive, were significantly more susceptible to *B. cinerea* than corresponding background lines, and all the mutants tested failed to express the *Trichoderma* ISR. Auxin-resistant mutants were mostly pathogen-affected similarly to their background and expressed ISR after *Trichoderma* soil treatment. Ethylene (E)-related mutants included E-insensitive, E-overproducers and E-reduced production mutants. E-insensitive mutants *ein2-1*, *ein-6*, *etr1-1*, *etr1-3*, E-overproducers *eto1-1*, *eto2* and E-reduced mutant *hls1*-1 were more susceptible to *B. cinerea* than WT, whereas other mutants did not differ from their background. The lack of ISR was observed in *ein2*, *ein3-1, ein4*, *ein5-1*, *eto1-1*, *eto3*, *etr1-3,* and *hls1-1*. All the gibberellin (GA)-related mutants were strongly affected by *B. cinerea*. GA-deficient mutants were more severely infected than GA-resistant or GA-insensitive ones. Two GA-deficient mutants tested (*ga1-4* and *ga2-1*) were unable to express *Trichoderma* ISR, whereas contradictory results were observed with GA-resistant mutant *spy3*. Among 12 pathogenesis-related mutants (including *cpr, eds, jar, npr*, *pad,* and *rps* -mutants) most mutants expressed ISR. Mutants *npr1-5*, *rps3-3* and *jar1* had low level of basal resistance to *B. cinerea*, and were found to be blocked in their ability to express *Trichoderma* related ISR.

In Arabidopsis, earlier work with strains of non-pathogenic rhizobacteria demonstrated Jasmonic acid (JA)- and ethylene-dependent ISR that is effective against different pathogens. In the present work, the mutant analyses showed similarity of the rhizobacteria- and *Trichoderma*-ISR signaling pathways in which components from the JA and ethylene response are engaged to trigger a defence reaction. However, not only JA and ethylene but also other phytohormones such as ABA and GA are involved in activating resistance mechanisms in *Arabidopsis*. Earlier work with T39 suggested other modes of action.

Trichoderma applications on sugar beet leaves reduce lesion size and sporulation of *Cercospora beticola* and increase sucrose yield

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Cercospora leaf spot, caused by *Cercospora beticola* Sacc. is considered one of the main sugar beet diseases in southern Europe, negatively affecting root yield and sucrose content. Genetic resistance for this character is partial and the control of the disease is mainly chemical, achieved through repeated fungicide applications. Today, there is a growing interest for alternative ways of control, to be used in integrated or biological crop systems. *Trichoderma* spp. are a class of biocontrol agents that showed effectiveness towards a number of plant pathogens. This study reports the evaluation of some *Trichoderma* isolates as biocontrol agents of C. beticola.

Some preliminary laboratory and greenhouse screenings and two years open field trials led to the selection of two *Trichoderma* isolates that were tested again in open field in 2003 under natural C. beticola inoculum in Northern Italy. The climatic conditions delayed the appearance of the pathogen, and the disease severity was below the usual level for this environment. Repeated foliar applications of the isolates did not reduce the disease incidence (0-9 KWS scale). Nevertheless, one isolate led to a sucrose yield not statistically different from that of the treated control, represented by two traditional sprayings of fungicide, and significantly higher than the untreated control. It also significantly reduced C. beticola sporulation per unit of necrotic area in a way similar to the fungicide. Both isolates and fungicide reduced single lesion dimensions, unlike the untreated control that showed lesions of almost double size. Root yield was not affected by any treatment. The study seems to confirm the effectiveness of *Trichoderma* foliar applications for controlling cercospora leaf spot of sugar beet, with a performance not different from chemical protection, at low-pressure conditions of the disease.

Isolation of fungicide-resistant mutants from cold-tolerant *Trichoderma* strains and their *in vitro* antagonistic properties

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Among 128 isolates of *Trichoderma,* five cold-tolerant strains were found to possess excellent *in vitro* antagonistic properties against plant pathogenic *Microdochium nivale, Fusarium culmorum, F. oxysporum* and *Pythium debaryanum* strains. From the 16 pesticides tested, 7 fungicides, copper sulphate, carbendazim, mancozeb, tebuconazol, imazalil, captan and thiram showed significant inhibition on the *Trichoderma* strains, the minimal inhibitory concentrations were approximately 300, 0.4, 50, 100, 100, 100 and 50 μg/ml, respectively.

Mutants resistant to copper sulphate, carbendazim, mancozeb and tebuconazol were isolated from a *T. viride* and a *T. aureoviride* strain by ultraviolet light mutagenesis. The cross-resistance capabilities and *in vitro* antagonistic properties of the mutants were determined in the presence of sublethal concentrations of distinct fungicides mentioned above. For instance carbendazim-resistant mutants showed total cross-resistance to benomyl and thiabendazole at a concentration of 20 μg/ml. A great number of fungicide-resistant strains were found to be potential candidates for application in complex integrated pest management.

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Application of a yeast, *Pichia anomala* strain WRL-076 to control *Aspergillus flavus* for reducing aflatoxin in pistachio and almond

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The Food and Agriculture Organization (FAO) estimates that 25% of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins. Contamination of aflatoxin in tree nuts, peanuts, corn and cottonseed has been recognized as a serious food safety hazard to both human and animal. The domestic and export markets of almonds, pistachios and walnuts presently allow a maximum level of aflatoxin contamination in the edible nuts to be 20 ppb. More recently aflatoxin levels of 2-4 ppb have been declared mandatory by importing European Countries. Even very low levels of infection of the nuts by *A. flavus* can result in aflatoxin levels above these mandatory standards. Numerous researchers from universities and USDA locations are conducting nationwide aflatoxin elimination and control studies, including breeding, genetic engineering, regulation of aflatoxin biosynthesis and microbial ecology. Managing aflatoxin contamination via biological control is a promising approach currently available.

A bioassay has been developed to screen for effective yeast inhibiting both the growth of the *Aspergillus flavus* and aflatoxin production. Several species of yeast have been identified as potential effective biocontrol agents. One particular yeast, *Pichia anomala* strain WRL-076 was tested further for its antagonistic activities to reduce spore production of toxigenic and atoxigenic isolates of *A. flavus* in pistachio flowers and nut-fruits as well as in almond and pistachio leaves. Both sterilized and non-sterilized plant materials were tested in lab experiments. The growth and viable number of *A. flavus* spores on yeast-sprayed and unsprayed pistachio fruits, leaves, flowers and almond leaves were monitored. Spore production of *A. flavus* was reduced by 60 to 80% on plant samples sprayed with *P. anomala* WRL-076 compared to the control samples without the spray of this yeast. Field evaluation of the efficacy of the biocontrol agent, *P. anomala* WRL-076 was carried out in an orchard. Pistachio nut-fruits on the tree were individually wounded with a dental needle and sprayed with aqueous suspension of yeasts at $3x10^7$ cells/ml in mid July. The wounded nut-fruits without yeast-spray were used as controls. Four weeks after the spray, wounded nut-fruit was hand picked from the tree and immediately placed to a special agar medium. The nut-fruits were incubated at 28^0C for eight days. Colonization and fungal growth on wounded nut-fruits were visually scored with the aid of a microscope. 95% of the wounded nut-fruits (control) showed obvious fungal growth. In contrast nut-fruits sprayed with the effective yeast showed much less fungal growth and 35% of the nut-fruits did not have any visible fungal growth. The viable fungal counts on individual nut were enumerated by standard microbiological techniques. Colonization of *A. flavus* in yeast-sprayed and wounded nut-fruits was lower than the control. Furthermore the total spore numbers of *A. flavus* on yeast-sprayed nut-fruits had decreased significantly in comparison to the control. The results indicate that *P. anomala* strain WRL-076 could protect the wounded nut-fruits from the infection and growth of *A. flavus* and other fungi. Using pistachio as a model system, similar efficacy of biocontrol activity of *P. anomala* WRL-076 antagonistic to *A. flavus* could be predicted for almond.

Screening of microorganisms and other alternative seed treatments for activity against seed-borne pathogens of cereals

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Historically, seed-borne pathogens have been one of the major obstacles in cereal production. In conventional agriculture where highly effective chemical seed treatments are regularly used, seed-borne pathogens are nowadays considered only a minor problem. However, in organic farming seed-borne pathogens are apparently becoming increasingly important. The main reason is the lack of effective methods of seed treatment allowed to be used in organic farming. We performed a screening under controlled conditions for activity against common bunt (*Tilletia caries*) of wheat with laboratory preparations of microorganisms belonging to different taxa (*Trichoderma*, streptomycetes and other bacteria), formulated microbial products, plant extracts and resistance inducing agents. Treatments with activity were observed in each of these groups, except in the group of resistance inducing agents. When effective treatments were repeatedly tested, a large variability was observed between the experiments. This was especially true in case of the bacteria, but less pronounced for the tested isolates of *Trichoderma* and *Streptomyces*. The most active treatments were included in a field trial and further evaluated in greenhouse tests for activity against other important cereal seed-borne pathogens (leaf stripe of barley, *Helminthosporium gramineum*; net blotch, *Drechslera teres*; *Fusarium*-seedling blight). The results will be presented and discussed.

Protease overproduction of *p*-fluoro-phenylalanine resistant and colony morphology mutants isolated from a *Trichoderma harzianum* strain with biocontrol potential

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Several *Trichoderma* strains have been reported to be effective in controlling plant diseases, and the action of fungal hydrolytic enzymes is considered as the main mechanism involved in the antagonistic process. Chitinolytic and glucanolytic enzyme systems involved in the mycoparasitism of *Trichoderma* strains have been investigated in details and are well characterised. The ability of *Trichoderma* strains to produce extracellular proteolytic enzymes is also known, however, proteases have not yet attracted the attention of studies in biocontrol.

Trichoderma harzianum T334 - a strain with the ability to produce low levels of proteases constitutively - is a potential biocontrol agent against plant pathogenic fungi. To improve its fungal antagonistic capacity, mutagenetic program was undertaken for the construction of protease overproducing derivates. The mutant strains were obtained by means of UV-irradiation and were selected for p-fluorophenyl-alanine (pFPA) resistance or altered colony morphology. It was revealed by means of specific chromogenic protease substrates, that both trypsin-like and chymotrypsin-like protease secretion was elevated in most of the mutant strains. Alterations in the posttranslational events related to the secretory pathway, changes in the membrane permeability, the derepression of the nitrogen metabolism and alterations in the cell wall biosynthesis are among the supposed events resulting in protease overproduction. Gel filtration chromatography profiles of trypsin- and chymotrypsin-like proteases were complex, suggesting the presence of more isoenzymes. Differences in the profiles between the mutants and the wild type strain were also detected. Certain mutants with increased protease secretion proved to be better antagonists against plant pathogenic Fusarium culmorum, Pythium debaryanum and *Rhizoctonia solani* strains in in vitro antagonism experiments. The method of UV-mutagenesis with the selection for pFPA-resistant or colony morphology mutants seems to be appropriate for the isolation of mutants with an increase in extracellular enzyme concentrations. Another potential advantage of this method is, that strains improved by mutagenesis can get registration for on field use more easily than those deriving from protoplast fusion or transformation. The results of the present study indicate that improved extracellular protease secretion may result in enhanced biocontrol activity of *T. harzianum*, and demonstrate, that strains with increased biocontrol effectiveness could be easily produced by simple UV-mutagenesis.

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Evaluation of *Trichoderma* strains as biocontrol tools for integrated management of strawberry root rot

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Strawberry root rot diseases are caused by a complex of pathogens, including *Rhizoctonia, Pythium* and *Phytophthora* that can seriously reduce plant vigour and productivity. These pathogens may be disseminated on infested plant material and may already be present in infested fields. The goal of our research group is to develop and implement an IPM system for managing strawberry root rots with emphasis on biological approaches and reduced pesticide use. The objective of this study was to evaluate the effectiveness of *Trichoderma* biocontrol strains in suppressing pathogens and promoting plant growth and productivity on transplants and in the field. Two *Trichoderma* strains with known biocontrol activity were tested: *T. harzianum* strain T22 and *T. hamatum* strain T382. Strawberry transplants were grown from naturally infested runner tips in greenhouse conditions. The tips were planted in potting mix amended with each biocontrol agent (2002 and 2003) and with a combination of both strains (2003 only); untreated potting mix was used as a control. Root rot severity and plant growth (leaf area, leaf and root dry weight) were assessed 4-5 weeks after planting on a subsample of transplants, and fungal isolations were made from roots. The remaining transplants were planted in field plots treated with compost, compost colonized with *T. hamatum* T382, a chemical fumigant, or untreated. Plants sampled throughout the growing season were assessed for root rot development and plant growth, and yield was determined. In the greenhouse, transplant treatments did not affect root rot severity in 2002 but all *Trichoderma* treatments significantly reduced root rot compared to the control in 2003. Transplants treated with T382 showed greater leaf area and root dry than the control in 2002 but no treatment differences were observed in plant growth in 2003. Root rot pathogens were isolated less frequently from transplants treated with *Trichoderma* than from untreated controls. Transplant treatments showed no effect on disease or plant growth under field conditions in 2002. Fumigation of field soil significantly reduced root rot severity and stimulated plant growth compared to the other treatments, while compost and compost+T382 offered no benefit compared to the untreated control. Marketable yield did not differ significantly among the fumigated, compost with T382, or control field soil treatments but was lower for compost alone than for the fumigated treatment. This preliminary study illustrates some of the challenges encountered in integrating *Trichoderma* biocontrol agents in strawberry transplant and fruit production systems.

Protoplast fusion, using nitrate non-utilizing (nit) mutants in the entomopathogenic fungus *Verticillium lecanii* (*Lecanicillium* spp.)

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Commercial biocontrol agents Mycotal and Vertalec have a high virulence against whitefly and aphid, respectively. Strain B-2, which was isolated from green peach aphid in Japan had high epiphytic ability on plant leaf surfaces, while Mycotal and Vertalec did not. In order to obtain useful new strains that had high virulence as well as colonization ability, protoplast fusion was done between three strains of *Verticillium lecanii*. Nit mutants were used to select the protoplast visually, to which the fusion had happened. The fusion experiment was attempted by combining nit mutants of complementary phenotype, and then 126, 44 and 4 fusion derived colony isolates were detected from combination of Vertalec×Mycotal, B-2×Mycotal and B-2×Vertalec, respectively. Furthermore, the phenotype of these fusion derived colony isolates was evaluated according to the colony morphology. As a consequence of this morphological characteristics were various compared to original isolates. We will discuss further genomic and pathogenicity analyses about these fusion derived colony isolates.

Establishment and survival of a *Trichoderma* mycoparasitic strain in some compost products

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Composting is a biological process in which organic biodegradable wastes are converted into a hygienic, humus-rich product, compost, used in agriculture as amendment to improve soil structure and promote plant growth. Compost has been shown to also have the potential to provide biological control against plant diseases caused by different microorganisms, particularly when used in container media. The suppressive effect seems to be related to indigenous microbial consortia. However, this effect is not stable, depending mostly on the origin and quality of the compost. To improve the stability and reproducibility of the biocontrol effect, selected strains of microbial antagonists have been added to composts. However, the success of antagonistic enrichment and durable survival depend on the compost type. With the final goal to promote the exploitation of compost in biological control strategies, we evaluated the establishment and survival of a mycoparasitic *Trichoderma atroviride* isolate in three compost products. Spent mushroom (*Agaricus bisporus*) compost derived from wheat straw-bedded horse manure taken after steaming at the end of the mushroom production process (young champost) or 3 months later (mature champost), green compost derived from fruit, vegetable and garden waste taken one month after heat peaking were enriched with a sporal suspension of a benomyl tolerant isolate of *T. atroviride* to obtain a concentration of $8x10^5$ conidia ml^{-1} product. Peat moss and potting soil were used as controls.

The population dynamics of the mycoparasitic fungus evaluated over a period of 140 days clearly showed that all compost products were suitable for the establishment of *T. atroviride*. Survival of the fungus was higher in mature champost and green compost than in young champost. In particular, over the experimental period mature champost was able to sustain the population level of *T. atroviride* obtained by enrichment at the beginning of the experiment. Moreover, this compost product promoted the highest levels of saprotrophic activity on cellophane disks and ability of the mycoparasitic fungus to colonise the root of wheat seedlings, two important properties for the success of *Trichoderma* species in the control of diseases caused by soil-borne fungi. The potential toxic effect of these compost products, enriched or not enriched with *T. atroviride* on the survival of the collembolan *Protaphorura armata*, a soil animal species used in ecotoxicological tests, was also evaluated. The products did not affect the survival of animals. No statistical differences were found among type of products or between enriched and unenriched product. In addition to adults introduced into the jars containing the products at the beginning of the experiment, live juveniles were also observed in enriched and unenriched products.

Pseudomonas fluorescens EPS62e, a potential biological control agent of fire blight

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Fire blight, caused by *Erwinia amylovora*, is a serious disease affecting several rosaceous plants with great commercial and economic interest. A biological control agent, *Pseudomonas fluorescens* EPS62e, was isolated from the surface of a pear fruit using a selective enrichment procedure. The strain was selected among 600 strains of *P. fluorescens* and *Pantoea agglomerans* obtained from flowers, fruits and leaves of rosaceous plants in a survey performed through several geographic areas of Spain. Strain EPS62e was evaluated for suppression of immature fruit, blossom and shoot infections, under controlled environment conditions, providing control levels similar to chemical control with copper or antibiotic compounds. The strain colonize and survive well in wounds on immature fruits, young leaves and flowers, and reduce significantly fire blight infections in these plant organs when applied in preventive treatments. Preemptive exclusion of the pathogen *E. amylovora* by surface colonization and nutrients depletion, and cell-to-cell interaction appear to be the main mechanisms of biocontrol. Specific methods of analysis of EPS62e were developed using strain-specific molecular markers based on sequence characterized amplified regions (SCAR). Several DNA amplified fragments, which differentiate EPS62e from other P. fluorescens isolates, were obtained using random amplified polymorphic DNA (RAPD). Three RAPD fragments were selected, cloned and sequenced for the design of SCAR primers. Two couples of SCAR primers gave unique and specific amplification products for EPS62e, whereas gave no amplification with 162 strains of *P. fluorescens* and 71 strains of other closely related species. These molecular markers offer a simple and unambiguously detection system for the biocontrol agent EPS62e suitable for monitoring and colonization studies.

Biocontrol agents against downy mildew of Grape: an ultrastructural study

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Plasmopara viticola (B. et C.) Berl. et De Toni, the grapevine downy mildew pathogen, causes an economically very important disease. It is particularly destructive in viticultural regions with warm, wet conditions during the growing season, including parts of Europe and North America. The aim of this work was to find and test biocontrol agents (BCAs) against *P. viticola* to limit the use of chemicals in organic agriculture. Several microorganisms were sampled from grapevine leaves in plants situated in an abandoned vineyard individuated in Tuscany (central Italy). The microorganisms were isolated culturing in PDA medium small leaf sections-to select only endophytes-, and maintaining in thermostat at 25°C. 126 isolates were then purified and finally tested against grape downy mildew according to the screening system described by Pertot et al., 2003. Among the 126 isolates, five fungal microorganisms inhibited the sporulation of *P. viticola* in vitro. They were identified as all belonging to *Alternaria alternata* species. An ultrastructural analysis was carried out to know the cellular interactions between the pathogen and Alternaria alternata in the grape leaf tissue. A test to demonstrate the main activity of Alternaria alternata against *P. viticola* was also performed.

Characterization of avocado root-colonizing bacteria antagonistic against *Rosellinia necatrix*

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The most important disease affecting avocado orchards in the south of Spain is the white root rot caused by *Rosellinia necatrix*. From the perspective of a modern, sustainable and environmentally friendly agriculture, one of the control strategies, which can be applied, is the use of microbial antagonists as biological control agents. In this sense, the antagonistic microorganisms must be ecologically adapted to establish and survive in the environment where it must exerts its action competing and avoiding growth of the pathogen. Biological control of phytopathogenic fungi is usually based on the production of anti-fungal factors, which has to be delivered at the right time and site by an agent capable to colonize efficiently the roots. Since rhizosphere competence is often the limiting factor for biocontrol, the aim of this project was to isolate and characterize a collection of bacterial strains, antagonistic to *R. necatrix*, capable of establishing and surviving in avocado roots. The root system of avocado plantlets obtained from *in vitro* germinated seedlings, was bacterized with bacterial suspensions previously extracted from roots of symptomless avocado trees located in orchards of the Andalusia coast affected by *Rosellinia necatrix*. After ten days of growth, root tips were macerated and plated onto King's B medium. After several days of incubation at 25ºC, bacterial colonies were isolated and initially characterized according to their morphology, biochemical and physiological tests, resulting in more than twenty different rhizosphere-competent strains. Subsequently, their *in vitro* antagonist ability against *R. necatrix* was determined, and several of the isolated strains inhibited fungal growth. We also analysed two characteristics known to be involved in bacterial colonization of roots: a) motility of the different isolates in solid media containing different concentration of agar (swimming, swarming and twitching), and b) the production of AHL molecules involved in regulation by quorum-sensing (QS). With this purpose, an *Escherichia coli* AHL-monitor strain based on the QS-regulatory system of *Vibrio ficheri* was used. So far, we have found that all of the antagonistic strains tested showed at least two types of motility and one of them also showed QS-regulation detectable by this monitor strain. Currently, we are in the process of determining which types of antagonistic compounds are produced by the antagonistic isolates, i.e. the production of lipases, chitinases, β-glucanases, proteases, antibiotics and volatile compounds. To confirm the role of these bacterial strains in the protection of avocado roots against the fungal pathogen *R. necatrix*, the selected bacteria will be used in bacterisation experiments to test biocontrol activity using an avocado/*Rosellinia* test system under growth chamber conditions. In such experiments, aerial and root symptoms will be recorded. Finally, the localisation on the avocado root of selected isolates and the pattern of colonization will be studied using Gfp-tagged derivatives of these strains by epifluorescence microscopy.

Effect of relative humidity on the efficacy of mycoparasitic fungi and antagonistic bacteria towards cucurbit powdery mildew

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Powdery mildew is one of the most important diseases of cucurbits worldwide. Disease induces as the most characteristic visual symptom a whitish, talcum-like, powdery growth developing on leaf surfaces, petioles and stems. On cucurbits the disease can be caused by two fungal species, *Golovinomyces cichoracearum* or *Podosphaera fusca*, however, to date *P. fusca* has been identified as the sole cause of the disease in Spain. Application of fungicides is currently the principal practice in most cucurbit crops for managing powdery mildew, therefore, it is important to explore and develop new control strategies. From the onset, environmental concerns always favour biological approaches, however, in terms of efficacy, chemicals are usually more potent than their biological counterparts. In the case of powdery mildew, relative humidity has been by far the most limiting factor to the effectiveness of biological control agents. Since powdery mildews have mostly an ectotrophic life cycle, one can assume that they can be easy targets for hyperparasites and antibiotic-producing microorganisms. In previous works we have reported the ability of two mycoparasitic fungi, *Ampelomyces quisqualis* (AQ10®, Ecogen) and *Lecanicillium lecanii* (Mycotal®, Koppert Biological Systems) and four bacterial isolates identified as *Bacillus* spp. to control powdery mildew disease by an *in vitro* test on detached leaves of melon. The aim of this work was to confirm the efficacy of those biological control agents under different regimes of relative humidity; thus, biocontrol trials on melon seedlings were carried out under controlled conditions of light, temperature and relative humidity (RH) in plant growth chambers. The mycoparasitic fungi were applied as curative treatments three days after *P. fusca* inoculation and their biocontrol activities were evaluated 12 days later. The best biocontrol results were obtained at 90-95% RH for both fungi, achieving disease reduction values of 80-90%, whereas below 80% RH disease reduction dropped to 62%. Similarly, when mycoparasites were applied together biocontrol results did not improve significantly with respect to each one alone. The bacterial inocula were composed by cells harvested from liquid cultures in stationary-phase of growth (48 hours) and were applied four h after pathogen challenge, recording biocontrol activities 16 days after inoculation. For all strains the best biocontrol results were also obtained at 90% RH, achieving disease reduction indexes of 80%, whereas at 75% RH disease reduction values ranged from 44 to 60%. Biocontrol results similar to those obtained with bacterial cells were observed when cell-free culture filtrates were tested, suggesting that among the different microbial activities possibly involved in the biocontrol action of these strains, antibiosis may have a major role. These results strongly highlight the need to carry out further research on the biocontrol effectiveness of these microorganisms under both field and greenhouse conditions prior to developing integrated control strategies including these agents to minimize the dependence on chemicals of cucurbit powdery mildew management.

Mycoparasitic activity of isolates of *Trichoderma* sp. against sclerotia of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum (Lib.) De Bary and *Sclerotium rolfsii* Sacc., with their wide host range, represent two of the most destructive pathogens on many economically important crops. These pathogens produce resting structures known as sclerotia which permit the fungi to survive in the absence of a host and have a strong resistance both to chemical and biological degradation. Sclerotia are composed of an outer rind layer of melanized cells that are thought to be responsible for resistance to microbial degradation in soil. Isolates of genus *Trichoderma* have long been known for their mycoparasitic ability against sclerotia of phytopathogenic fungi. One hundred twenty isolates, belonging to the Fungal Collection of the Department of Fruit Science and Plant Protection of University of Pisa, were screened for their mycoparasitic ability against sclerotia of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum.* Putative mycoparasitic *i*solates belong to the *Pachybasium*, *Longibrachiatum*, *Saturnisporum* and *Trichoderma* Section and included *T. asperellum*, *T. atroviride*, *T. aureoviride*, *T. crassus*, *T. effusum*, *T. erinaceum*, *T. fasciculatum*, *T. flavofuscum, T. hamatum*, *T. harzianum*, *T. helicum*, *T. hunua*, *T. koningii*, *T. minutisporium*, *T. oblungisporium*, *T. polysporum*, *T. sinensis*, *T. stromaticum*, *T. velutinum*, *T. virens, T. viride*, *Hypocrea rufa* and *Trichoderma* sp., with different geographic origin and isolated from different matrix. An isolate of *Clonostachys rosea* was also tested. Some species were represented by only one strain. Tests were performed by plating ten sclerotia of *S. rolfsii* and *S. sclerotiorum* on Petri dishes containing potato dextrose agar colonized by each isolate. Plates were replicated twice. After seven (*S. rolfsii*) and 14 (*S. sclerotiorum*) days of incubation at 24°C, firmness of sclerotia was evaluated. Soft sclerotia were counted and discarded, the remaining were sterilized in a solution of ethanol (50%) and NaClO (1% active chlorine), washed, plated on PDA and incubated at 24°C. After one week sclerotia were observed to detect the presence of antagonists. Sclerotia that gave rise to a colony of the antagonist were recorded as infected. Mycoparasitic activity was expressed according to the percentage of decayed or infected sclerotia. Distribution of mycoparasitic ability according to the taxonomic position of *Trichoderma* isolates was evaluated.

Strategies to provide integrated biological control of late blight of potato to replace copper for sustainable organic agriculture production

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Late blight (caused by *Phytophthora infestans*) is one of the most important diseases affecting organic and conventional potato production worldwide. Under suitable environmental conditions for the pathogen, the disease can spread very rapidly and can cause complete crop losses. Protective copper fungicides, which are currently used to control the disease in most organic production systems, are estimated to extend the length of the growing season by between 10 to 30 days. However, the amounts of copper fungicides allowed to be used in organic farming in the EU have recently been drastically reduced, and a complete ban is foreseen for the future. Therefore, alternative control treatment strategies are required.

For the control of some other fungal pathogens alternative treatments have already been developed, including microbial antagonists and also plant extracts, which have an effect on the fungus by direct antifungal activity, by stimulation of competitive microorganisms and/or by inducing plant resistance. In the framework of the EU-funded project "Development of a systems approach for the management of late blight in EU organic potato production – Blight-MOP" a screening with over 100 natural substances, including microorganisms and plant extracts, was carried out targeted to *P. infestans.* In addition, and in order to increase the control potential against *P. infestans*, compatible combinations of microorganisms and plant extracts were established. The tests were done on detached potato leaves and potted plants. In further studies in semi-field trials, optimal application intervals and persistence of the substances were tested. Application of the bacterial antagonist *Xenorhabdus bovenii* at two-day intervals resulted in good control of late blight. These methods, observations and results are reported here as attempts to further explore and develop integrated biological control methods for sustainable organic agriculture potato production.

Experiences with the entomopathogenic fungus *Beauveria brongniartii* for the biological control of the common cockchafer *Melolontha melolontha*

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Melocont®-Pilzgerste, a commercial product based on barley kernels colonised by *Beauveria brongniartii*, was tested against the common cockchafer *Melolontha melolontha* in large field trials over a period of eight years. The barley kernel product was applied in pastures with a slit seeder at various times of the year. The highest efficacy of the product was achieved by incorporating the inoculum into the soil at a depth of 3 to 10 cm. A threshold of inoculum in soil was required to ensure epidemic levels in the pastures (>2 x 10^4 spores g^{-1} dry weight of soil). The results of field trials conducted between 1995 and 2003 with the barley kernel product indicated that the density of *B. brongniartii* increased continuously after each of the applications as long as the target pest was present. With the exception of a site with sandy-textured soil, fungal populations within soils persisted above the threshold level. Re-isolation studies showed a uniform vertical distribution of fungal spores at depths of 10 to 20 and 20 to 30 cm, respectively.

Microsatellite marker analysis confirmed that the applied strain and re-isolated strains were identical. The application of the *B. brongniartii* barley kernel product resulted in a sufficient suppression of cockchafer populations after only 2 years of applications (>20 % prevalence of mycosis), and populations were reduced from > 70 larvae / m^2 to less than 22 larvae/m^2 after 5 years. After that, no relevant damages by *M. melolontha* have been reported at treated sites. The described biocontrol agent was registered by the Austrian plant protectant legislation as of June 2000 based on the results of this study.

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Investigation of *Trichoderma* strains isolated from winter wheat rhizosphere

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Species in the fungal genus *Trichoderma* are of great economic importance as sources of enzymes, antibiotics, as plant growth promoters, xenobiotic degraders, and most importantly, as commercial biofungicides. One hundred forty *Trichoderma* strains were isolated from roots of winter wheat growing in agricultural fields of southern Hungary, and the diversity of species was examined based on morphological, biochemical and molecular characters. Morphological data were collected based on structure and measure of conidiophores, phialides and conidia. Differential utilization of a total of 100 carbon- and 45 nitrogen sources was applied as a biochemical marker. For investigation of molecular diversity, a cellulose-acetate electrophoresis mediated isoenzyme analysis was used. After initial testing of 12 enzymes for activity and resolution of bands, 6 of them (glucose-6-phosphate dehydrogenase, glucose-6-phosphate isomerase, 6-phosphogluconate dehydrogenase, peptidase B, and phosphoglucomutase) proved to be appropriate for analysis of the full sample set. Comparing the different electrophoretic types, each of the enzymes applied could be used as molecular markers in the identification of *Trichoderma* spp. Some *Trichoderma* spp., which are new in Hungarian habitats, were successfully detected in our investigations.

Latest results on the biocontrol of fire blight in Germany

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Research on alternatives to the antibiotic streptomycin for the control of fire blight on pome fruits, caused by *Erwinia amylovora*, is of great importance for German and European fruit growing. Several industrial and experimental products for biological control have been developed in recent years. For example, an antagonistic preparation, BIOPRO, showed a control efficacy of up to 60% or the plant extract from *Hedera helix* revealed a high efficacy in the field in combination with a low concentrated copper compound and metal salt. The control of fire blight was comparable with the antibiotic streptomycin under artificial and natural infection conditions. Moreover, a natural product based on an etheric oil of *Thymbra spicata* (Biozell 2000B), which was developed together with Turkish scientists from the University of Akdeniz, Antalya, showed good effect under field conditions.

Efficacy of control agents on powdery mildew: a comparison between two populations

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Powdery mildew (*Sphaerotheca macularis* f.sp. *fragariae*) causes severe losses in strawberries both in traditional cropping systems in Mediterranean climates and in greenhouse soil free systems, which are more common in central Europe and in some areas of Northern Italy. Most fungicide sprays applied to strawberry are targeted at control of powdery mildew. However, routine sprays are no longer acceptable and only high quality fruit can be marketed. Alternative approaches to disease control are aimed at developing integrated disease management that include non-chemical fungicides and biocontrol agents. The aims of the study are: to test several alternatives to chemical fungicides of strawberry powdery mildew and to compare two populations of *S. macularis*, one from a temperate climate (Northern Italy) and the other form a Mediterranean climate (Israel) in their sensitivity to the control agents. A wide selection of BCAs (*Ampelomyces quisqualis*, *Trichoderma harzianum* T39, *Bacillus subtilis*, *Bacillus* sp. F77, *Cladosporium tenuissimum*), chemical fungicides (azoxystrobin, kresoxim methyl, penconazole, hexaconazole, miclobutanil, tetraconazole, triadimenol, nuarimol, fenarimol, pyraclostrobin + nicobifen, mancozeb + famoxadon, pirifenox, pirazofos, polyoxin Al, sulphur, sulphur + pinolene, ultrafine oil), plant extracts (Milsana – an extract of giant knotweed, neem oil, Agribioprop – a plant extract), resistance inducers (acylbenzolar-s methyl, harpin), salts and mineral nutrients (potassium bicarbonate, sodium bicarbonate, potassium phosphate monobasic, calcium carbonate, iron chelate, mineral nutrients solution) were tested in conidial germination tests. The rate of germination and germ tube length were measured on glass slide and strawberry leaves. Biomass of the hyphae produced by the germinated conidia was calculated. The comparison of the two populations (Israeli and Italian) regarding several parameters (germination, biomass and germ tube length) was carried out in respect to susceptibility of the powdery mildew to a selection of the listed control agents. The control results related to the different control agents were grouped according to the nature of the agents. Several control agents were more effective on leaves than on glass (acylbenzolar-s methyl, harpin, *T. harzianum* T39 and *Bacillus* sp. F77). Salts better influenced germ tube elongation than germination. All the chemical fungicides were effective in inhibition of germination and/or the rate of germ tube elongation. The chemicals that had no significant effect on germination were tetraconazole and triadimenol, but they reduced germ tube elongation. Kresoxim methyl was the only chemical that had a different effect in Italy if compared with Israel, probably because of the presence of resistant strains in Israel and not in Italy, where it is not yet registered for the use on strawberry. The role of the plant tissue in the mechanism of action of some biocontrol agents and resistance inducers can be hypothesised. Although RAPD PCR analysis of genomic DNA with arbitrary primers and apPCR with primers specific to microsatellites show that pools of isolates randomly collected in Italy and Israel were different, the Italian and Israeli *S. macularis* populations can be considered similar in sensitivity to the tested control agents, except for chemicals that are already known to select resistant strains.

Antagonistic activity of entomopathogenous fungus *Beauveria bassiana* against pathogens: perspective of combined use against insects and fungi

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The pathogenic activities against several species of insects of some fungi belonging to *Beauveria* genus are well known. Some are already used as commercial products in organic and integrated pest management of several crops. A partial antagonistic activity has been reported also against some fungi and bacteria. A combined activity against insects and fungi could be of added value to pest and disease control relaying on BCAs.
In a dual culture test screening employed for detecting antagonistic activity against *Armillaria mellea* and *Penicillium digitatum,* a wild strain of *Beauveria bassiana* (strain B1) isolated from dead insects (*Corythucha ciliata*), has been selected. In an early interaction stage the B1 isolate seems to have antibiotic activity against the two pathogens, but at a later stage hyperparassitic activity against *A. mellea* was observed. *P. digitatum* was less inhibited than *A. mellea* and it even showed a slight antagonistic activity against B1. The side effects of B1 on predatory mites have been evaluated.

Biocontrol agents and their integration in organic viticulture in Trentino, Italy: characteristics and constrains

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Demand for organic products is continuously increasing in Italy and Europe. In Trentino region, organic viticulture is also increasing, responding to the request of the market. Organic viticulture aims at improving products (wine and table grape) quality by avoiding the health and environmental risks that are due to chemical pest management. Pest control in organic agriculture has to be managed with a holistic approach, and biocontrol agents (BCAs) are a useful tool both against diseases and insects. The use of BCAs in organic pest management and the present situation of organic viticulture in Trentino has been documented with a survey done in the collaboration with ATABIO (organic growers association of Trentino). The objective was to evaluate the incidence and severity of diseases in biologic vineyards and the existing problems and constrains in the non-chemical control of grapevine pests.

The highest diffussed variety is Chardonnay, followed by Pinot gris and Pinor noir with about the same percentage and Cabernet sauvignon, Mueller Thurgau; in the Adige valley Merlot, Marzemino are the more diffused varieties. Few other minor and local varieties are also present mainly in new plantations. Downy mildew resistant varieties are not present. Downy mildew is the main problem, followed by powdery mildew and mites. Gray mould is a problem only in the Pinot gris variety, but can be managed with agronomical techniques. BCAs (*Trichoderma harzianum*, *T. viride*, *Ampelomyces quisqualis*) are not used and disease control is usually achieved with copper and sulphur (10-17 treatments in total, with an average of 10-12), mainly because these treatments are considered easier to apply, cheap and effective. Mating disruption is used to control vine moths. There is an increasing worry about the possible diffusion of Flavescence doreé from the near region (Veneto) and treatments against *Scaphoidaeus titans* are done with pyrethrum, where this vector is present. BCAs against insects (*Bacillus thuringiensis, Beauveria bassiana*) are, similarly to diseases, scarcely applied for the same reasons. Weeds are a big problem in the early stages of new vineyards. Growers are generally satisfied about public extension service even if there is a common demand for more attention to organic farms. The information on diseases and pest management is derived mainly from books and specialised papers. Other growers or private consultants are not an important source of information. Ideological motivations or personal decision guided farmers to start an organic farm or convert it from IPM.

In conclusion, apart some problems at the beginning of a new plantation or during the conversion from IPM to organic management, pest and diseases are not severe constrains for organic growers in Trentino and they are mainly controlled with sulphur, copper, pyrethrum and mating disruption. BCAs would be more frequently applied if information on their application and efficacy will be available from valid experimentation.

Grapefruit extract inhibits sporulation and development of *Phytophthora ramorum* on rhododendron

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Phytophthora ramorum discovered first time in Germany and Holland in 1993 – 94 and described by Werres et al. (2001) is one of the most serious threat of Californian oaks causing sudden oak death (SOD) and rhododendron twig blight. Some other ericaceous plants, *Viburnum* species and at least 20 other plant species are hosts of that pathogen. The species produces zoosporangia and chlamydospores already 2 – 3 days after inoculation of rhododendron leaves and may develop at temperature from about 2 to 30º C with optimum at 20ºC. Phosetyl Al alone or in mixture with fenamidione strongly suppressed the development and spread of the pathogen. In this study activity of grapefruit extract (GE) as the active ingredient of Biosept 33 SL was evaluated. Amendment of soil leachate with GE at conc. 8 µg/cm³ strongly inhibited zoosporangia formation. At dose 40 µg of GE/cm³ zoosporangia were not produced. Low concentration of GE did not suppressed chlamydospore formation of *P. ramorum*. Increase the dose of GE to 200 µg/cm³ completely inhibited their formation in soil leachate. Application of GE at conc. 165 µg/cm³ as rhododendron sprays 3 h before inoculation of plants with *P. ramorum,* inhibited the spread of necrosis on leaf blades and stems about twice.

Control of *Phytophthora cryptogea* with *Trichoderma viride* combined with furalaxyl and chitosan

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Phytophthora foot rot of gerbera (*Gerbera jamesonii* Bolus), caused by *P. cryptogea* Pethybr et Laff., is one of the most dangerous disease of that plant grown under covering. The pathogen is also known as the causal agent of root and stem rot of pot flowers and field grown ornamental nursery plants. Losses caused by *P. cryptogea* varied from a few % sometimes even to 60%. Chemical compounds are mostly used for the disease control. Previous study of Orlikowski showed, that *Trichoderma* spp. and *Gliocladium roseum* might suppress the development of *P. cryptogea*. In this study activity of *Trichoderma viride* isolates, obtained from diseased gerbera as well as furalaxyl and chitosan toward *P. cryptogea* was studied *in vitro* and in greenhouse trials. In Petri dishes bioasseys *T. viride* inhibited strongly the growth of *P. cryptogea*. Mycoparasitism, mediated by intimate hyphal interaction was observed. Adhesion of the mycoparasite hyphae to the pathogen caused a serious degradation in cytoplasm. Amendment of PDA medium with furalaxyl already at dose 8 μg/cm³completely inhibited the growth of *P. cryptogea* culture. In case of chitosan only slightly inhibition of *in vitro* colony growth was observed even when compound was used at 200–1000 μ/cm³. Amendment of peat with *T. viride* spores resulted in the decrease of *P. cryptogea* population density at least 35%. The period of *T. viride* application had significant influence on healthy stand of gerbera. Amendment of peat with the mycoparasite 10 days before substratum infestation with the pathogen was the most effective. Furalaxyl applied as plant drench immediately after gerbera planting almost completely controlled *Phytophthora* foot rot. Combined application of *T. viride* and furalaxyl decreased population density of *P. cryptogea* but this effect was not as good as single used of the chemical. Chitosan applied 2 weeks before amendment of peat with *T. viride* resulted in the decrease of cfu number of the pathogen about 50% and increased the gerbera healthy stand to 60%.

Survey of antagonistic yeasts occurring in apple trees managed with different production systems

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Apples are grown in Brazil using conventional, integrated and organic production systems. Since different management practices could affect the beneficial microflora growing as epiphytes on the crop, this research aimed to compare the frequency of yeasts which are antagonistic to the two main apple pathogens: *Colletotrichum gloeosporioides* and *Penicillium expansum*. Yeasts with antagonistic properties against the first pathogen were surveyed in the three production systems and those antagonistic to the second one, were obtained from apple trees growing in the conventional and in the integrated system. The isolates were selected from fruit washings and morphologically characterized. Selection of antagonists was done by spraying unwounded apples with a suspension containing both the pathogen and each yeast isolate when *C. gloeosporioides* was used or on wounded apples when *P. expansum* was studied. Results showed that in both the integrated and ecological systems, the frequency of yeasts antagonistic to *C. gloeosporioides* was similar and higher than in the conventional system. Yeasts antagonistic to *P. expansum* were also more frequent in integrated than in conventional system. Many antagonistic organisms were selected with potential to control both pathogens suggesting that yeasts must be protected on apple orchards.

Integrated management of *Pythium* root rot in flower bulb production

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Root rot caused by fungi of the genus Pythium is an important disease in flowerbulb cultivation in the Netherlands. Measures to control this disease such as fungicides do not always result in consistent disease control. In this study, several control methods are tested in single and combined applications, to investigate compatibility and synergism of these methods. Control methods, which are being studied at this moment, are antagonistic *Pseudomonas* bacteria, biofumigation, fungicides and cultural measures.

Antagonistic *Pseudomonas* spp. strains producing the antibiotic 2,4-diacetylphloroglucinol (Phl) or producing biosurfactants showed Pythium root rot control of different bulb crops (*Iris, Crocus, Hyacinthus*) in pot experiments under controlled conditions. A biosurfactant-producing strain almost completely prevented Pythium root rot in *Hyacinthus* and *Crocus*. A mutant strain, lacking the biosurfactant-production, did not suppress root rot in *Hyacinthus* indicating that this biosurfactant compound is involved in disease control. In bioassays under field conditions both the biosurfactant-producing strain and a Phl-producing strain were able to control disease of different crops (*Hyacinthus*, *Iris* and *Crocus*). In several expanded field trials both strains were able to control of Pythium root rot but these results vary per field and year to year. Furthermore we are investigating the possibility of using a biofumigation crop to reduce Pythium inoculum in the soil before planting the bulbs. So far we have obtained promising results under field conditions.

Since several measures tested in single applications do not result in sufficient Pythium root rot control we are developing an integrated strategy to manage this disease. At the moment integrated control of Pythium is investigated in field experiments with naturally infested soil. In these field experiments single and combined applications of *Pseudomonas* spp. strains, a biofumigation crop and standard and lower dosages of a pesticide are compared.

Laboratory and field evaluation of epiphytic vine yeasts against sour rot of grapes caused by *Aspergillus carbonarius*

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Recent research data indicate that *Aspergillus niger* and *A. carbonarius* are the main sour rot agents of grapes in the Mediterranean countries. Both fungal agents are very dangerous for raisin and wine production due to the formation of the mycotoxin ochratoxin A. In order to evaluate the efficacy of vine yeasts in controlling sour rot, caused by *A. niger* and *A. carbonarius* several isolates were selected from a large collection of epiphytic vine yeasts collected from vineyards of various regions of Greece. In the laboratory tests, individual berries were dipped in yeast suspensions (10^7cfu/ml) after being wounded by piercing a hole of 2mm in diameter. After 24 hrs the berries were inoculated with *A. carbonarius* (10^4 conidia/ml). After five days of incubation (Rd 100%, temp. 22 C, photoperiod 12h) it was demonstrated that the rot was inhibited by 60%-80% compared with the untreated control.

The two most effective isolates were selected for further experimentation. One of them designated as GY-18, originated from Xinomauro variety from central Macedonia of Greece was identified as *Cryptococcus laurentii*. The second one, designated as K-4, originated from Robola variety Cephalonia island has not yet been identified. Isolates, GY-18 and K-4 along with the fungicides switch and copper oxychloride (cupravit 50 WP) were tested in vineyard trials of Nemea region. Chemical or yeast evaluation was focused on the effectiveness of sprays on identification and quantification of *A. carbonarius* and *A. niger* population and on the percentage of bunches showing sour rot symptoms at harvesting time. As for the effect of applications on population synthesis and quantification at harvesting, it was shown that untreated control berries were infested with *A. carbonarius* and/or *A. niger* by 80%, while the corresponding figures were 33% for switch, 55% for GY-18, 49% for K-4 and 70% for cupravit. Percentage of infected bunches by *A. carbonarius* and *A. niger* reached 33% in the untreated control plots. The corresponding figures were 14% for switch, 15% for GY-18, 12% for K-4 and 23% for cupravit. The reported data indicate effectiveness of switch as chemical control and yeasts as biocontrol agents against sour rot.

Selection of entomopathogenic nematodes for heat tolerant and desiccation traits

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Entomopathogenic nematodes (EPNs) are currently used in biological control of insect pests, but their sensitivity to environmental constraints reduces their application possibilities. Thus there is the need to find natural EPN strains that are able to survival to environmental stress. In this study we present the selection for heat tolerance under laboratory conditions of Dauer Juveniles (DJs) larvae of five EPN strains. Two of the five strains showed heat tolerance ability since more then 75% of their DJs survived after 30 minutes exposure at 40°C. The two selected strains were also tested for their ability to survive- to low humidity conditions. Surviving was assessed after exposure of nematodes to different relative humidity (RH) levels in sealed desiccators. DJs were able to survive several hours in absence of water in liquid state in a chamber with 100% relative humidity. Additionally, the two strains were able to survive at 85% RH treatment for two hours. The advantages of using heat and desiccant tolerant entomopathogenic nematodes in biological control program are discussed.

Integrated control of blue mould on apple with biocontrol agents, chemical alternatives and heat treatment

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Recently, different strains of the yeast *Metschnikowia pulcherrima* were isolated from apple carposphere and studied for their efficacy and mode of action. All of them are effective against *P. expansum* on apples with a varying degree of control and act through competition for nutrients and/or space, without producing toxic metabolites *in vivo*. Biological means cannot at the moment solve all the problems of postharvest rots. Different physical (hot water dipping) and chemical control methods (acibenzolar-S-methyl (ASM), ethanol, or sodium bicarbonate) could be used together with the application of the strain BIO126 to obtain more consistent results. The antagonist, applied at 10^8 cells ml^{-1}, proved to be the key element for the control of blue mould, resulting more efficient after cold storage, with a reduction of 56.6% of the lesion diameter. Heat treatment and sodium bicarbonate significantly improved the efficacy of the biocontrol agent with storage at 23°C.

To study the possibility of a single application of the biocontrol agent (10^7 cells ml^{-1}) with reduced dosages of sodium bicarbonate or ethanol, the viability of the microorganism with these chemicals was studied and a new set of experiments was established. Higher reductions of the lesion diameter were obtained simply treating with the biocontrol agent. Significant results were provided also by the application of 20% ethanol or 5% sodium bicarbonate before the biocontrol agent and by the application of BIO126 in 0.1% sodium bicarbonate.

The application of the cell suspension of BIO126 *M. pulcherrima*, preceded or not by a pre-treatment with sodium bicarbonate or ethanol, could become a successful alternative to fungicide usage in postharvest disease control of pome fruit. The fungistatic effect of ethanol or sodium bicarbonate could be associated to the effect of the biocontrol agent, persistent on the fruit for long periods.

Comparison of wood colonisation by Alpine *Phlebia gigantea* strains, Rotstop® and *Trichoderma* treatments in a pure Norway spruce stand in Alpine environment

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Biological control of *Heterobasidion* root rot is a common silvicultural procedure in North Europe. One of the most commonly applied protectants is Rotstop®, a commercial preparation based on a *Phlebia gigantea* strain isolated from Finland. In previously conducted field tests in the Alps, Rotstop® has performed quite well as a biological control agent of *H. annosum* in spruce forest stands. Because of the specific environmental and ecological characteristics of the Alps region, the adaptability and suitability of selected local strains of *P. gigantea* was now compared with Rotstop® and other fungi used for biological control.

The field experiment was carried out in a pure Norway spruce (*Picea abies*) stand in Trentino, North-Eastern Italian Alps. Rotstop®, eight Italian strains of *Phlebia gigantea* (four from Trentino, two from Central Italy and two from South Italy), and two strains of *Trichoderma viride* (one from Finland, one from Tuscany) were applied onto healthy spruce logs. Wood disks were cut from the logs after two growing seasons in autumn 2001. The number of colonies of all antagonistic fungi and the percentage of wood infected were determined from both sides of the disks. Among the treatments, Rotstop® proved to be the second best in terms of both the number of colonised logs and the percentage of colonised surface. *P. gigantea* strains from Trentino showed variable ability to colonise spruce wood, with one strain showing significantly higher wood colonisation than Rotstop®. Among the Italian *P. gigantea* strains, the ones from Trentino showed significantly higher wood colonisation than the strains from Central and South Italy. In all cases *P. gigantea* strains colonised most of the sapwood and occasionally also the hardwood. The Italian *Trichoderma* strain gave results comparable to those obtained with the *P. gigantea* strains from Trentino. However, the Italian *Trichoderma* strain showed very different colonisation ability than the Finnish one. These data indicate that there is considerable variation in the wood colonisation ability among strains belonging to the same species. The impact of biodiversity of antagonistic strains on the control of *H. annosum* is discussed.

In vitro antagonism of *Armillaria* spp. against *Heterobasidion annosum* s.l.

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Species of *Armillaria* and *Heterobasidion* are root-rot fungi that often occur in the same forests and colonize same conifers. From the point of view of forestry, *Heterobasidion* is generally more harmful whereas some common *Armillaria* species are weak pathogens only and do not attack vigorous trees. In stumps and weakened trees they are competitors of *Heterobasidion* and may thus restrict its spreading in the stand. In this preliminary work mutual relationships between different species of *Armillaria* and *Heterobasidion* were investigated on malt extract agar medium. This is the first time that *Armillaria* is used in antagonism test against *H. annosum s.l.*

Four diploid isolates of *A. borealis, A. cepistipes, A. gallica, A. mellea* and *A. ostoyae* were paired *in vitro* in dual cultures with four heterokaryotic isolates of *H. annosum s.str., H. parviporum* and *H. abietinum*. In general, *Heterobasidion* was the stronger partner and grew slowly into the *Armillaria* colony. However, some *Armillaria* strains were antagonistic and stopped its growth. This antagonism seems to be a strain-specific rather than a species-specific property; however, the most strongly pathogenic *Armillaria* species, *A. ostoyae,* showed to be the weakest antagonist. There were no clear differences in the behaviour of different *Heterobasidion* species. Would be interested to assess the relationships of *Armillaria* and *Heterobasidion* spp. in a further wood blocks experiment.

Selectivity and effectiveness evaluation of Electrolyzed Acid Water (EAW), alone and in mixture with Shin-Etsu surfactants, against *Phytophthora infestans* (Mont.) De Bary, the causal organism of late blight on tomato

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Electrolyzed Acid Water (EAW), generated by electrolysis from tap water and potassium chloride (KCl), is a mean of high level disinfection similar to that already used in medical sector for endoscopes reprocessing.
Thanks to the extremely high efficacy on a wide range of fungi, bacteria and viruses the possibility of its application in agriculture should be investigated for the reduction of conventional chemical treatments.
With this aim, first laboratory trials were carried out in 2003, applying EAW, alone and in mixture with Shin-Etsu surfactants, in order to assay its preventive (2003/EAW/01, 02) and curative fungicide activity (2003/EAW/03, 04, 05, 06, 07, 08) against *Phytophthora infestans* (Mont.) De Bary, the causal organism of late blight on tomato.
The commercial standard fungicides compared were copper-based.

Poster Session 2

Serratia plymuthica HRO-C48: Strategy to control *Verticillium dahliae* in oilseed rape and effects on the microbial community

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Verticillium dahliae Kleb is a soil-borne fungus causing vascular disease that results in severe yield and quality losses in many crops. In the last years yield losses in oilseed rape production caused by *V. dahliae* became of alarming relevance worldwide. Till now, there exists no possibility to control the pathogen due to the lack of potent fungicides. In previous works, the effect of *Serratia plymuthica* strain HRO-C48 to control *V. dahliae* and to promote plant growth of strawberry was shown. The objective of the study is to develop a strategy to use the biocontrol capacity of *S. plymuthica* for protecting oilseed rape against Verticillium wilt.

Theoretically, seed coating or infiltrations are possibilities to apply *S. plymuthica* cells on oilseed rapeseeds. Therefore, these treatments were used to evaluate rhizosphere competence, disease reduction, plant growth promoting effect and the influence on the community of autochthonous micro-organisms in both field and greenhouse trials. Rifampicin-resistant cells of *S. plymuthica* were reisolated from the rhizosphere in abundances of 10^2 - 10^6 CFU rfw g^{-1}. To estimate the effect of the applied bacteria on the microbial community the culture-independent method of Single-Strand-Conformation-Polymorphism analysis of PCR-amplified fragments of the 16S rDNA using universal and *Serratia* specific primers was used. Minor changes caused by the *S. plymuthica* treatment were detected. Observed shifts based mainly on the individual plant and the growth stage.

These studies were carried out in cooperation with e-nema GmbH (Raisdorf, Germany) and Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (Hohenlieth, Germany).

Biocontrol of avocado white root rot by using the bacterial strain *Pseudomonas fluorescens* pcl1606

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One of the most important diseases on avocado trees (*Persea Americana*) in Southern Spain field crops are the phytopathogenic fungus *Rosellinia necatrix* and the oomycete *Phytophthora cinnamomi*, causing the diseases avocado root rot and white root rot respectively. Control of *P. cinnamomi* is possible by combined control actions using different strategies as chemical and physical control, the use of tolerant rootstocks and application of biocontrol agents. Otherwise, control of the white root disease of avocado is especially complex, because until now, only solarization showed protective results against *R. necatrix*, and other approaches, like chemical fungicides or tolerant rootstocks still experimental. In relation with biocontrol strategies, the use of *Trichoderma* spp. showed good results in greenhouse experiments, but still variable on field tests. Few other approaches have been done to use other microorganisms as biocontrol agents. For this, the use of bacterial strains as biocontrol agents could be an alternative treatment that can improve the integrated management of white root rot.

The strain *Pseudomonas fluorescens* PCL1606 has been selected in previous studies because its antagonistic activity on plate assay against a very broad spectrum of phytopathogenic fungi and the oomycete *P. cinnamomi* and because its biocontrol activity against *R. necatrix* on greenhouse experiments. Moreover, detailed studies about the antagonistic activity of *P. fluorescens* PCL1606 showed that this bacterial strain produces a novel antibiotic compound (NAC) with a high antifungal activity that could be involved in biocontrol activity. In order to study the role of this antibiotic in the biocontrol activity, mutants impaired in the production of the antifungal antibiotic were constructed by transformation with the plasmid pRL1063a, which harbour a Tn5 transposon with the reporter genes *lux*AB. From 4.700 transformants, 7 different mutants impaired in the production of NAC have been obtained and characterized for their growth in minimal media, biochemical characteristics, and detection of the NAC by TLC experiments. Then, biocontrol experiments have been performed using the experimental avocado/*Rosellinia* test system, were the mutants constructed from the wild type strain *P. fluorescens* PCL1606 showed reduction of the protection levels against *R. necatrix*. The reduction in biocontrol activity is not complete, suggesting that more than one trait (production of antifungal metabolites) could take place in the biocontrol activity of the *P. fluorescens* strain. Simultaneously, sequencing of the flanking regions where the Tn5 insertion takes place in some mutants, showed that some of the disrupted genes presented homology to growth factors or transport membrane proteins.

Growth promotion and Induced resistance of tomato by rhizobacteria in calcareous soil of Uzbekistan

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Inappropriate application of mineral fertilisers and pesticides in crop and vegetable production has resulted in pollution and Stalinization of Uzbekistanian soil. In such regions to improve plant productivity with biological methods, like bacterial inoculants is an important approach. PGPR can help the improvement of plant growth, plant nutrition, root growth pattern, plant competitive responses to external stress factors, inhibit soil borne plant pathogens, induce plant resistance. The objectives of our research were, to isolate native plant growth-promoting bacteria from the calcareous soil, to evaluate their potential to control *Fusarium* dry rot of tomato disease plant growth. *Fusarium* dry rot is considered a serious threat to tomato plants under greenhouse conditions in Uzbekistan. Soil used in this study was calcareous calcisol. Tomato (*Lycopersicon esculentum*, cv. Leningrad) was employed as the inoculation experiments. The bacterial strains *Arthrobacter simplex ArS43*, *Bacillus amyloliquefaciens BcA27, Pseudomonas aureofaciens* PsA5, *P. denitrificans* PsD6 were used for the experiments. To carry out the pot experiments autoclaved sterile soil and non-sterile soil were used. The fungal inoculum was thoroughly mixed with soil in sterile plastic bags and then introduced in pots. Afterwards the plants were transplanted and inoculated with a bacterial suspension. Recently 30 bacterial strains isolated, obtained from calcareous soil and 9 strains were screened for their ability to suppress the growth of *Fusarium oxysporum* in vitro and to control dry rot development on tomato plants. The fungal pathogen strongly inhibited by *Bacillus* and *Pseudomonas* species, which has resulted in the appearance of inhibition zones. After screening, antagonist bacterial strains tested for their ability to control *Fusarium* dry rot of tomato in pot experiments. Tomato plants inoculated with *Fusarium oxysporum* started to show typical dry rot symptoms by the 7 day. Our results showed, that 2 strains *Pseudomonas* sp. RW12 and RW14 showed a high level of *Fusarium* dry rot control in vivo. After inoculation with *Bacillus* sp. RC14 and *Pseudomonas* sp. RW14 there were no symptoms of disease in inoculated plants. Damaged plants were lower in not sterile soils than sterile soils. Bacterial strains also effect on seedling growth of tomato in pot experiments. The strain *B. amyloliquefaciens* BA27, *Ps. aureofaciens* PsA5, *Pseudomonas sp*. RW12, RW14, and *Bacillus* sp. RC14 increased shoot and root length of tomato plants. The effect of bacterial strains on plant growth was higher in non-sterile soils to compare with sterile soil. Analysis of data obtained show that bacterial strains have significant effect on plant growth and disease control on greenhouse conditions with calcareous soil. All bacterial strains were salt tolerant and heat resistant that have abilities to survive in stressed environmental conditions.

Screening of bacteria and fungi for their Antagonistic activity against plant pathogens

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Biological control of seedling disease using non-pathogenic fungi and bacteria has received increasing attention. Several biocontrol agents, including bacterial strains *Bacillus, Pseudomonas* and the fungus *Aspergillus*, *Penicillium*, *Trichoderma* have been shown to antagonize seedling and root pathogens of pea, cotton, several different plants to various degrees from soil-borne fungal pathogens. The objectives of this study were: to isolate bacterial and fungal strains from the soil under cotton, to screen the isolates for their antagonistic activity towards a fungal root pathogen *Fusarium* species, and to evaluate their potential in biological control *Fusarium* seedling damping-off disease. One hundred thirty six (136) strains of *Trichoderma*, *Aspergillus*, *Penicillium* and 30 strains of *Bacillus*, *Pseudomonas, Arthrobacter,* and *Mycobacterium* were used for study. Fungi and bacteria were isolated from calcisol soil under grown cotton of Surhandarya region Uzbekistan by the serial dilution-spread-plate technique. Pure colonies were screened for antagonism toward plant pathogenic fungi *Fusarium* species. The Regional Collection of Microorganisms provided phytopathogenic species of *Fusarium solani f. argillaceum, F. javanicum, F. moniliforme, F. moniliforme f. lactis, F. moniliforme f. subglutinans, F. oxysporum,* and *F. culmorum.* Fungi spread on PDA plates. The ability of bacteria isolates to reduce *Fusarium* damping-off disease in cotton seedling was evaluated. Ten cottonseeds were placed in petri dishes with filter paper. Infected *Fusarium* seeds inoculated with antagonist bacteria were incubated at 23° C for 7 days and examined for evidence that seedling disease was inhibited by the bacterium. From the seven hundred forty tree (743) fungal isolates one hundred thirty six (136) were antagonists against the plant pathogenic fungi. Isolates of *Aspergillus ochraceoroseus*, *Trichoderma lignorum*, *Penicillium notatum, A. terreus*, and *A. ustus* showed more antagonistic activity among all isolates (24-33%). One isolate *A. ochraceoroseus* strain 1384 was a strong antagonist against *F. solani var. argillaceum* (22%) and *F. moniliforme var. lactis* (11%). *T. lignorum* strain 1171 and strain 158 were more antagonist (10 - 27 mm) against all other tested pathogen *Fusarium* species. Other species ex. *Penicillium, Aspergillus* showed lower antagonistic activity. The bacterial selection consisting of 10 strains of *Pseudomonas*, 8 strains of *Bacillus*, 6 strains of *Arthrobacter*, 3 strains of *Mycobacterium*, 2 strains of *Rhizobium* were evaluated for antagonism *against F. culmorum* and *F. oxysporum*. *Pseudomonas* isolates had more antagonistic activity then other bacterial isolates. Seed bacterisation with all of bacteria isolates increased seed germination (100%) over that of the control (80%), the root length and the number of lateral roots. Seedling leaves increased up to 90-100% compared to 30% for control. Seedling *Fusarium* damping-off disease was reduced in about 50% for cotton seedlings inoculated to all tested bacteria strains. This study has demonstrated that the strain has a potential to increase the growth of cotton seedling and to protect them from *Fusarium* root disease in calcareous calcisol soil.

Molecular characterization of biocontrol agents

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Biocontrol agents, under certain circumstances, have been shown to be as efficient as chemical fungicides for controlling plant diseases. Several species of *Trichoderma* are known mycoparasites and have been used successfully against soilborne pathogenic fungi. One aim when isolating *Trichoderma* strains is to identify those which can be used in new agricultural applications, and at present several are either being or have been registered as fungicides in various countries. Amongst the many requirements within the registration application, it is necessary to identify, characterise and assign names to the strains being registered. In the past, it was not uncommon that biocontrol strains were defined as *T. harzianum* in the sense of Rifai (1969) due to the limited classification system of the genus *Trichoderma*. However, today this is not the case and *Trichoderma* taxonomy is much more developed. *Trichoderma* along with *Fusarium* and *Aspergillus* is among the three genera with the largest number of ITS sequences described. *Trichoderma* is probably the only genus where every described species has been sequenced and for which a sequence can be found in GenBank. For biocontrol strains to be registered and used commercially it is necessary that they are accurately characterised. The present study was undertaken to determine the genetic diversity existing in a collection of strains, selected as biocontrol agents against phytopathogenic fungi during more than ten years. *Trichoderma* strains were collected from different geographical locations and culture collections.

Using the most recent taxonomic information available and sequence data obtained from the ITS1 region and a fragment of the *tef1* gene, biocontrol isolates of *Trichoderma* were analysed. Sequence alignments were generated using the CLUSTALX 1.81 program and phylogenetic analysis was carried out using PAUP, Version 4.0 (Phylogenetic Analysis Using Parsimony, Sinauer Associates, Sunderland, Mass.). Neighbor-Joining (NJ) trees were constructed for each data set (ITS1 or *tef1*) using the Kimura-2-parameter distance measure. The sequence analysis of the PCR fragments pertaining to these two genes easily discriminated biocontrol strains from virulent biotypes within *Trichoderma* genus. More than half of the biocontrol strains were grouped with the ex-type strains of *T. harzianum*, *T. inhamatum* or *T. virens* and 36% of the strains studied grouped with *T. asperellum*, *T. viride*, *T. atroviride* or *T. koningii*. Only 10% of the strains were located within *T.* sect. *Longibrachiatum*. Amongst the *Trichoderma* strains analysed in this study, no significant correlation was found between geographical origin or ecological niche and isolate taxonomy.

Studies of soil and rizosphera bacteria to improve biocontrol of avocado white root rot caused by *Rosellinia necatrix*

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In Europe, Andalucía (Spain) is the main area growing avocados, with more than 8.000 has of avocado orchards. Avocado root rots are the most important diseases in commercial plantations of this area. These diseases are caused mainly by the oomycete *Phytophthora cinnamomi* and the fungus *Rosellinia necatrix*. Control of avocado root rot disease caused by *P. cinnamomi* can be performed successfully by combinations of chemical fungicides, solarization, the use of tolerant rootstocks to the oomycete, and by application of mulches and antagonistic bacteria to the soil. On the other hand, control of white root rot is complex and at this moment, the only treatment resulting in some protection of avocados against *R. necatrix* is soil solarization, meanwhile effective chemical and biological control still experimental. The objective of our study is to improve in the biological control of the disease isolating and analysing rhizobacterial strains as potential biocontrol agents, especially based in their antagonistic activity, and further developing different strategies to applied this agents.

Bacterial isolation from soil and rhizosphere samples were carried out from different healthy avocado trees placed into affected areas by *R. necatrix*, located in Andalucía, resulting in 126 different bacterial isolates selected basically according to differences in colonies characteristics. After isolation in pure culture, these isolates were tested in a plate assay for antagonism against *R. necatrix* and *P. cinnamomi.* The bacterial strains showing fungal growth inhibition after five days at 24ºC were considered as antagonistic strains. Simultaneously, these antagonistic strains were basically differentiated using few tests: Gram stain and cell morphology, production of fluorescent pigments on King's B medium under ultraviolet light, oxidase test, glucose metabolism and arginine dihydrolase. The isolated antagonistic strains will be studied for their production of antifungal substances, as ezoenzymatic activities, volatile compounds and/or antifungal antibiotics.

The analysis of the bacterial strains results in 21 different bacterial strains having strong antagonistic activity against *R. necatrix* and *P. cinnamomi*: 11 bacteria strains were antagonistic only to *R. necatrix*, two of them only antagonistic to *P. cinnamomi* and eight bacterial strains were antagonistic to both pathogens. From these strains, nine bacterial antagonistic isolates showed fluorescent pigment in KB media under ultraviolet light, which indicate that these bacteria could be included into the *Pseudomonas* genera.
Initially, biocontrol tests are being carried out with antagonistic and other non-antagonistic bacterial strains using plants from "in vitro" germined avocado embryos to select the most protective strains. Roots from several two months-old plants will be bacterized with bacterial suspensions and them will be sowed in potting soil infected with *R. necatrix*. Survival of the plants and aerial symptoms will be recorded every day during the biocontrol experiment. The most protective strains resulting for these bioassays were are use in the greenhouses studies.

Replacement of copper fungicides in organic production of grapevine and apple in Europe (REPCO)

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The objective of our recently started project, partly funded by the European Commission (Framework 6; 501452), is to contribute to replace copper fungicides in organic agriculture by new measures for control of downy mildew (*Plasmopara viticola*) in grapevine and scab (*Venturia inaequalis*) in apple. Both major European organic crops strongly depend on copper fungicides. Permitted amounts will be reduced stepwise after 2006 (Council Regulation (EEC) 2092/91, Annex II) to avoid environmental risks. In European countries where copper fungicides are already out of use, production of organic apples suffers severe economical problems because of insufficient scab control. Potentiators of resistance, organically based fungicides and biocontrol agents will be screened and evaluated in grapevine and apple. The risk of pathogen evolution during use of novel control measures will be estimated to allow the development of sustainable strategies. Effects of crop management practices in organic agriculture on overwintering of *V. inaequalis* will be assessed. Novel disease control measures and knowledge will be integrated into organic management systems. 'Pipeline' products already under development elsewhere will be included and where necessary optimised in their use.

Evaluation of population density of *Pichia anomala* strain K and *Candida oleophila* strain O and their protection against *Penicillium expansum* Link on apples

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The application of *Pichia anomala* (strain K) and *Candida oleophila* (strain O) in practical conditions had previously offered a high protection against *Penicillium expansum* only when an antagonist density of 10^4 cfu/cm^2 was detected on the intact surface of fruit. According to this, the initial concentrations of application in controlled conditions for each antagonistic yeast were defined in order to obtain this level of 10^4 cfu/cm^2. Whatever the strain studied, the initial application concentration of 10^8 cfu/ml allowed obtaining a density of yeast on the intact surface of apple higher than 10^4 cfu/cm^2 after 24 hours, whereas a density lower than 10^4 cfu/cm^2 was observed when an initial application concentration of 10^5 cfu/ml of apple surface is used. Three scenarios reflecting the practical conditions of biological application were then tested with different periods of incubation between biological treatment, wounding of fruit surface and pathogen inoculation. Whatever the strain, the initial application concentration of 10^8cfu/ml allowed to obtain the highest densities of yeast's per wound and the highest protective levels in comparison to the use of initial application concentration of 10^5 cfu/ml. The protective levels were positively correlated with the density of yeast determined in the wounded sites. Furthermore, these protective levels were influenced by wounds humidity. The protective levels registered on wet wounds ranged between 52 and 100 % while those observed on dry wounds did not exceed 30 %. Whatever the scenario used, the yeast density per wound and the protective levels induced by strain O were higher than those observed for strain K.

Biological Control of Powdery Mildew by Q-fect WP® (*Ampelomyces quisqualis* 94013) in Various Crops

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An isolate, *Ampelomyces quisqualis* 94013, was selected as a biocontrol agent for the control of powdery mildew in various crops. It was formulated as a wettable powder and termed Q-fect WP ® by Green Biotech Co. Ltd. in Korea. The Q-fect WP ® was treated onto plants of eight crops to control powdery mildew in the greenhouse. The occurrence of powdery mildew in the crops was investigated 7 days after treatment of the Q-fect WP ®. The control value of powdery mildew by the commercial product was 67.7% in strawberry, 75.2% in cucumber, 74.8% in melon, 88.9% in tomato, 86.2% in *Ligularia fischeri*, 77.1% in gerbera, 68.5 % in rose and 70.0% in grape. The results showed that the Q-fect WP ® could be used effectively as a biofungicide for controlling powdery mildew in the eight crops. The technical grade of *A. quisqualis* 94013 was registered as a biofungicide of cucumber powdery mildew in March 2003 and the commercial product will be granted in Korea in early 2004.

Exploitation of spent mushroom compost in biological control against plant diseases: preliminary results

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Spent mushroom (*Agaricus bisporus*) compost (SMC) pasteurised or re-composted to remove harmful organisms at the end of mushroom production process, has been shown to improve soil structure and promote plant growth. SMC seems to provide also a biocontrol effect against some plant diseases caused by soil-borne fungal pathogens. Compost enriched with suitable antagonists could increase the level of suppressive effect and thus promote the exploitation of this organic product in biological control strategies. Previous studies carried out in our laboratory showed that mature SMC was suitable for the establishment of a mycoparasitic *Trichoderma atroviride* strain (TA312). However, high Electric Conductivity (EC) and pH are limiting factors for using SMC as plant substrate. SMC, to decrease EC, could be leached with water or treated with chelating agents; unfortunately the cost of these treatments is at present too high. Lignosulphonates (LS) are by-products of the acid sulfite pulping process. They are low cost products, with low pH and chelating activity; in particular, they are efficient for sequestering metallic ions. Million of tons of LS are yearly produced and are mostly incinerated. Moreover, some *in vitro* tests showed that LS have also a direct biocontrol effect against several soil-borne fungal pathogens. However, phytotoxic effects have also been described.

In this study we evaluated the effect of Calcium-LS (Ca-LS) added (1%) to SMC on some chemical, biochemical (fluorescin diacetate {FDA} hydrolysis rate and β-glucosidase and chitinase activity) parameters and microbial population densities of such a product. The potential phytotoxic effects of SMC enriched with Ca-LS were also tested on *Lepidium sativum* plants. Finally the suppressive effect of SMC, SMC + Ca-LS, SMC + TA312, and SMC + Ca-Ls + TA 312 against the disease caused by *Fusarium oxysporum* f.sp. *melonis* was also evaluated under controlled conditions. Peat moss was used as control. Our results indicate that the addition of Ca-LS to SMC didn't affect the chemical parameters and population densities of indigenous total aerobic bacteria, actinomycetes and total fungi. Microbiological activity, measured as FDA hydrolysis rate and chitinase activity, was similar in SMC and SMC + Ca-LS. Whereas β-glucosidase activity was higher in SMC + Ca-LS than in SMC. No phytoxic effects of SMC+ Ca-LS were observed. Melon plants grown in peat-based substrate amended with SMC (30% v:v) and inoculated with *F. oxysporum* f. sp. *melonis* propagules one week before transplanting, were significantly less attacked by the pathogen than plants grown in absence of SMC. The enrichment of SMC with Ca-LS and TA 312 prior the inoculation in to the plant growing mix significantly increased the suppressive effect of the substrate.

An antifungal β-1,3-glucanase (AGN13.2) from the biocontrol fungus *Trichoderma asperellum*

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Trichoderma species have been investigated as biological control agents for over 70 years due to the ability of several *Trichoderma* strains to antagonize other filamentous fungi, including many plant pathogenic species. Mycoparasitism is one of the main stages involved in this process. In this study, we report on the antifungal activity of an exo-β-1,3-glucanase (glucan 1,3-β-glucosidase [EC 3.2.1.84], namely AGN13.2, from the antagonistic fungus *Trichoderma asperellum* T32, against the strawberry pathogen *Botrytis cinerea* and the avocado pathogen *Rosellinia necatrix*. The compatibility of AGN13.2 with other purified hydrolases and fungicides has also been tested. Antifungal assays against *B. cinerea* were performed including potato dextrose broth, conidia suspension and enzymatic extract in microwell plates. To determine the effects of the enzyme on fungal germination, the enzymatic solution was added simultaneously to the spore suspension. To determine the effects of the enzyme on hyphal growth, the enzyme solution was added after most spores were germinated. The area covered by mycelium was estimated as relative values with the program Q-Win (Leica Microsystems) modified for this particular application. Antifungal assays against *R. necatrix* were performed in Petri dishes supplemented with PDA and inoculated in the centre with a 5 mm diameter mycelial disk of pathogenic fungus. Enzyme solution was added over the disk on each fungal plate. After different times the diameter of *R. necatrix* growth was recorded. Sterile bidistilled water was used as negative control for these assays. Each experiment was repeated twice on separate days and included three replicates. All data were combined for statistical analyses. Regarding fungal germination of *B. cinerea*, no effects were detected after the addition of AGN13.2 by itself and combined with other hydrolytic enzymes. However the ED50 value on *B. cinerea* spore germination for fungicide captan was reduced from 0.35 mg/mL to 0.12 mg/mL by adding 10 μg/mL of AGN13.2. Germ tube elongation of B. cinerea was inhibited by the mixture of an endochitinase (CHIT42), a β-1,6-glucanase (BGN16.3) and a β-1,3-glucanase (AGN13.2). Regarding *R. necatrix*, only preliminary results have been obtained. The antifungal activity of AGN13.2 suggests that this enzyme contributes to the antagonism of *T. asperellum*. In addition to its possible medical applications against dental caries, important agricultural applications can be envisaged for this enzyme.

Can incomplete spatial coverage of control measures prevent invasion of fungal parasites?

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Whilst protection of each host in a plant population prevents invasion, such a complete spatial coverage is often not achieved. In particular in soils, where the root system needs to be protected from invasion, the delivery of a control agent to every susceptible root is seldom possible. Even at the field-scale it may be desirable to reduce the area over which a control measure is applied, for example to slow the rise of fungicide resistance or if economic costs are high. This raises the question what fraction of susceptible hosts needs to be protected to prevent an epidemic? In this paper we evaluate empirical evidence and theoretical arguments for the existence of spatial thresholds to control invasion of the soil-borne fungus *Rhizoctonia solani* in heterogeneous populations of substrates. The work is centred on the hypothesis that the invasion of soil-borne fungi in populations of substrates is analogous to the spread of infection through discrete host populations or fields with susceptible crops. For such spatial systems, *percolation theory* predicts that if a critical fraction of susceptible hosts is protected, invasion stops. To test this hypothesis, we use artificial but replicable microcosms in which the spread of *R. solani* is quantified through a population of agar sites on a lattice. A random fraction of sites is removed as an analogue of locally successful but spatially incomplete control. Analysis of fungal growth in the microcosms revealed the following epidemiologically important features: *i*) removal (e.g. via protection) of a *critical fraction* (60%) of susceptible sites is sufficient to *prevent invasion* into a population that otherwise would have been invaded; *ii*) removal of susceptible sites introduces a *shield* preventing a large proportion of the population from being invaded; *iii)* with an increasing number of protected sites, the remaining sites are less well connected forming a tortuous network with bottle necks resulting in a reduced rate of invasion, but with *occasional out-bursts* of fungal spread. We conclude that there is a threshold for spatial coverage of a control measure above which invasion is prevented. We conjecture that this holds for a broad class of organisms spreading through heterogeneous environments within crops. This means that not all plants have to be protected by a biocontrol agent in order to prevent invasion of a pathogen. The extent at which a control strategy is applied spatially can therefore be a critical component of disease management.

Occurrence of breakdown in the biocontrol of crown gall disease by the *Agrobacterium radiobacter* strain K84 in Italy

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Biological control of crown gall by using the *Agrobacterium radiobacter* strain K84 represent the most successful application of biological control for preventing plant diseases worldwide. Crown gall induction is due to the transfer of a DNA fragment (T-DNA) containing the oncogenic genes, from the tumor inducing plasmid (pTi) of tumorigenic bacteria to the plants where it is integrated and expressed. Biocontrol activity of strain K84 is mainly performed through the production of a bacteriocin, the agrocin 84 that is coded by the conjugative plasmid pAgK84. The biocontrol efficacy is very high on peach plants and some ornamentals that are mainly infected by biovar 2 tumorigenic strains that are sensitive to agrocin 84.

The selection of pathogenic recombinants insensitive to the antagonist may determine a breakdown in the biocontrol of crown gall in the field. Dangerous recombinants take origin through plasmid exchanges that may occur between the antagonist and tumorigenic agrobacteria. In 2001 a breakdown in the biocontrol of crown gall disease was reported in an Italian nursery of peach plants where K84 was used to protect the rootstocks. Agrobacteria strains were isolated from the tumors and from the soil around the tumors (tumorosphere) of the galled plants and identified. Molecular studies were performed to determine the plasmid content and the chromosomal characteristics of the isolates in order to identify the type of plasmid exchange and the genotypes involved in the failure of the biocontrol agent. Transconjugant strains were isolated both from gall tissues and from tumorosphere and all were originated by the transfer of pAgK84 to virulent and avirulent autochthonous agrobacteria. The analysis of pathogenetic characteristics and root colonization ability of recombinants showed that these strains are very competitive in soil and may represent a real threat to the application of K84.

The use of the *tra*$^-$ deletion mutant strain K1026 that is a derivative of strain K84, should prevent the risk of pAgK84 transfer into pathogenic *Agrobacterium* strains and safeguard the efficacy of *A. radiobacter* for biological control of crown gall. Its use should be promoted wherever strain K84 is used to control crown gall.

Influence of wheat seed treatment with *Clonostachys rosea* on the expression of PR proteins

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Pathogenesis-related (PR) proteins are involved in plant systemic resistance against pathogen attacks, moreover they may be stimulated by non pathogenic microorganisms. The possible involvement of *PR1*, *PR3* (chitinase), *PR4* (wheatwins), *PR5* (osmotin) and *PR9* (peroxidase) proteins and genes in wheat systemic resistance induction by *Clonostachys rosea* 47 (*CR47*), an antagonist of *F. culmorum* (*FC*), has been investigated. Wheat seeds were coated with *CR47* and four days old coleoptiles were artificially inoculated with a plug of ten days old *FC* colony. Seedlings were evaluated for disease symptoms 24, 48, 72 and 96 hr after inoculation; at each time, seedlings and roots were harvested and either RNA or proteins were extracted. Peroxidase and chitinase activities were determined after isoelectrofocusing (IEF) in 5-8 and 3-10 pH ranges and after SDS-PAGE in the presence of glycol chitin, respectively. PR4 proteins were detected after immunoblot analysis carried out using anti-wheatwin1 polyclonal antisera, whereas expression levels of *PR1*, *PR4* and *PR5* genes were evaluated by RT-PCR using specific primers. Peroxidase activity increased with the time and it was more evident in roots than in coleoptiles. A peroxidase isoform (PI close to 7.2) was slightly induced by *FC* inoculation in coleoptiles, but it was reduced by *CR47* treatment; its activity was highly enhanced when *CR47* seed treatment was followed by *FC* inoculation. Another peroxidase isoform (PI close to 8.2) showed in roots a similar behaviour. Concerning chitinase activity, no differences were found between treated and untreated samples after SDS-PAGE separation. Transcripts corresponding to wheat *PR4* genes were expressed at very low levels in both coleoptiles and roots harvested from control seeds remaining unchanged throughout the period of sampling. On the contrary, transcript expression was induced in the same tissues upon *FC* infection either in control or *CR47*-treated seeds. A rapid accumulation of *PR4* proteins in all samples was evidenced, displaying an induction pattern similar to that observed at the transcriptional level. Besides, the steady-state levels of *PR1* and *PR5* genes were high and comparable to the corresponding controls in all tissues tested indicating that they are constitutively expressed. An induction of resistance in wheat coleoptiles with the antagonist along with the pathogen can be hypotized, as spatial and temporal *CR47* seed application and *FC* inoculation were different.

Possible involvement of induced systemic resistance in sugar beet against C*ercospora beticola* by leaf treatment with *Trichoderma* sp.

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The effect of the *Trichoderma* isolate BA12/86 (ISCI collection) on the response of defence of sugar beet against *Cercospora beticola* has been studied. Sugar beet cultivars (cvs) Duetto and Aaron (susceptible to *C. beticola*), Ritmo and Faro (low susceptible to *C. beticola*), at six leaves stage were treated with a ten-day old liquid culture (diluted 5 times in PDB, $16.6x10^6$ cfu/ml) of BA12/86. *Trichoderma* treatment was applied on one leaf of each plant and *C. beticola* ($1x10^5$ conidia/ml) was inoculated on non treated leaves (T+I) of the same plant, two days after the treatment. Non treated - non inoculated (NT-NI), non treated - inoculated (I) and treated - non inoculated (T) plants, served as controls. The experiments were carried out on 9 plants for each experimental group, under greenhouse conditions and repeated twice.

Three, four, five and seven days after inoculation, the non treated leaves were excised and plant proteins were extracted. Peroxidase and chitinase activities were respectively determined after IEF in 3-10 pH range and after SDS-PAGE in polyacrilamide gel containing glycol chitin. A high acidic peroxidase isoform (pI close to 3.6) was present in T+I plants: its activity, three days after inoculation, was higher in low susceptible cvs than in the susceptible ones, whereas, four-five days after inoculation (activity peak), it was more evident in susceptible cvs than in the low susceptible. This isozyme activity, since it was absent or very low in NT-NI, I and T plants, seemed to be due to an interaction between treatment and inoculation. Two chitinases (40 and 45 kDa) were present, with variable intensity, in protein extracts from each experimental group of Aaron cv, while a third chitinase, with higher molecular weight (50 kDa), gradually accumulated in T+I plants. The fact that there is a spatial and temporal separation between BA12/86 application and *C. beticola* inoculation, suggests a possible involvement of an induced systemic resistance in sugar beet plants.

Microorganisms associated with *Platanus acerifolia* W. growing in areas infected by *Ceratosystis fimbriata* f. sp. *platani*

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Platanus acerifolia, the hybrid of *P. occidentalis* and *P. orientalis* widely used for landscaping and for difficult growing sites, is highly susceptible to *C. fimbriata* f. sp. *platani* attacks. This fungus, introduced in Europe in the middle of the last century from North America, has high level of homogeneity in the Italian population, with the same genotype throughout the country. Moreover, it is a pathogen with a broad range of host species and the causal agent of the widely reported disease called canker stain of plane trees. Chemical control has proved ineffective against canker stain. At present, the genetic improvement of *Platanus* with the production of hybrids or the selection of resistant clones seems to offer a valuable strategy of countering the disease. During in *vitro* explant cultivation, obtained from healthy plants of *P. acerifolia* W. growing in infected areas, we observed the presence of bacterial populations in three explants showing an enhanced growth. The bacterial populations were isolated from the basal portion of the three explants using different media. The bacteria were subsequently identified on the basis of their metabolic profile (i.e. BIOLOG) and molecular traits (RAPD and 16S rDNA sequences). Studies are in progress to elucidate the possible effects of these bacteria used as biocontrol agents in areas infected by *C. fimbriata* f. sp. *platani*, as well as their role in the *in vitro* propagation of *Platanus* plants.

Seed treatments for organic vegetable production

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Due to the difficulty in organic farming of producing pathogen free seeds, and the lack of simple, effective non-chemical methods for seed sanitation, a substantial part of the seed used by European organic vegetable growers is still derived from conventional production. This will be strongly restricted after the year 2003 (EU Council regulation 2092/91). In March 2003, a EU-project "Seed Treatments for Organic Vegetable Production" (QLRT-2002-02239; STOVE) was initiated. The project is aimed at improving currently available, non-chemical methods for control of seed-borne vegetable pathogens and to develop new methods, which are acceptable to organic farming. The participants are Federal Biological Research Centre for Agriculture and Forestry (BBA), Germany (M. Jahn, E. Koch, C. Kromphardt, A. Schmitt); Plant Research International (PRI), Wageningen, Netherlands (S. Groot, J. v.d. Wolf); Swedish University of Agricultural Sciences (SLU), Uppsala, Schweden (G. Forsberg, B. Gerhardson); University of Gothenburg, Sweden (T. Amein, S. Wright) - Findus R&D AB, Bjuv, Sweden (R. Stegmark, M. Wikström); Nunhems Zaden BV, Haelen, Netherlands (J. v.d. Berg); Nunhems Zaden (Hild), Marbach, Germany (M. Mistele, S. Werner); University of Turin, (Agrinnova) Italy (M. Gullino, F. Tinivella); Horticulture Research International (HRI), Wellesbourne, Great Britain (S. Roberts). Together with three physical methods (hot water, hot air and electron treatment), microorganisms and other agents of natural origin acceptable to organic farming will be included in the project. Initially, the three physical methods are being adapted for different vegetable species (e.g. carrot, parsley, cabbage, lamb's lettuce, basil, bean) and their respective seed-borne pathogens (e.g. *A. dauci*, *S. petroselini*, *X. campestris*, *P. valerianellae*, *Fusarium* spp., *C. lindemuthianum*). In parallel, potential alternative seed treatments (micro-organisms, plant extracts, inducers of resistance) are also being tested. The efficacy of the methods will be compared in glasshouse and field trials, and selected combinations will be evaluated. Special regard will be placed on physiological factors determining the sensitivity of seeds towards the physical methods.

The results of the first experiments will be presented. (See http://www.stove-project.net/ for further information).

In vivo investigations on the efficacy of some antagonistic yeasts on lettuce plants against *Botrytis cinerea* pathogen

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The Authors make a commented presentation of the pathology of *Botrytis cinerea* - fungus isolated from grapevine and artificial inoculated with spores on lettuce and showed results regarding the efficacy of *Saccharomyces chevalieri* (antagonistic yeasts) used as biological control agents against *B. cinerea* attack on lettuce plants. Bioproducts based on antagonistic yeasts have been tested as suspension of *Saccharomyces chevalieri* spores (2,5 x 10^5; 1 x 10 ^{6} and 1,75 x 10^6 conidia/ml) and as conditioned products: Saccharopulvin 25 PU in two formulations (F_2 and F_2). The results obtained in experiments with artificial inoculated variants with *B. cinerea* conidia, showed that the attack levels on lettuce plants were: 82.5, 87.5 and 90.0% that, directly depending of spore concentrations: 2.5 x 10^5; 1 x 10 ^{6} to 1.75 x 10^6 conidia/ml. Using *Saccharomyces chevalieri* yeasts as suspensions for treatment resulted in the highest efficacy (E = 82.8%) in variant with the highest concentrations of spores (1,2 x 10^6 conidia/ml), being followed by variant with 6,0 x 10^5 spores/ml (E=80.0%); the efficacy was better than procimidon that is used as standard ethalon (E=74.3%). Using *S. chevalier* yeasts as conditioned products, the results obtained were highly significant in protection of lettuce plants against grey mould (E=90.9% at concentration of 2.5 x 10^5 spores/ml for Saccharopulvin 25 PU (F_2), followed by Saccharopulvin 25 PU (F_1) with the efficacy value of E=87.9 %). Saccharopulvin 25 PU in two concentration (F1 = 2x10^6 cfu/ml; F2 = 6x10^6 cfu/ml), was effective (average between 87.9 – 86.1% for F_1 and 90.9 – 88.9% for F_2) in protection of lettuce against grey mould comparing with chemical standards Benlate 50 WP and Ronilan 50 WP (45.4 – 47.2% for benomil and 69.7 – 75.0% for procimidon - less efficient in controlling grey mould). From two concentrations of Saccharopulvin 25 PU, the higher one (F_2 = 6x10^6 cfu/ml) was more efficient in controlling grey mould (E = 88.9 – 90.9%). The bioproduct Saccharopulvin 25 PU, based on an isolate of *S. chevalieri*, applied in greenhouse at a rate of 0.2%, proved good efficacy to control lettuce grey mould under artificial conditions of disease evolution. The relationship *in vivo* between *B. cinerea* and *S. chevalieri* showed a strong antagonism of the pathogen attack on lettuce plants.

Variability of β-tubulin gene as an indicator to the characterization of intraspecific variation in Japanese isolates of *Verticillium lecanii* (*Lecanicillium* spp.)

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Recently, it was suggested that a major part of the species formerly classified in *Verticillium* sect. *Prostrata*, especially *V. lecanii* and *V. psalliotae*, be transferred to *Lecanicillium* and that *V. lecanii* was subdivided into three species and renamed *L. lecanii*, *L. muscarium*, and *L. longisporum* according to morphological observations and PCR-RFLPs of ITS, mtDNA and β-tubulin (Zare and Gams, 2001). In order to clarify whether *V. lecanii* of mainly Japanese isolates were identified with *Lecanicillium* spp., DNA polymorphism in Bt-1 region of β-tubulin gene of *V. lecanii* isolates from insects, phytopathogenic fungi and other substrates were analysed by PCR-RFLP. The size of the PCR product from this region was 540 bp in all isolates used in this study except for one isolate (560bp). Enzymatic digestions of this region with *Alu* I, *Cfo* I, *Hinf* I, *Hae* III were detected several fragment pattern, reflecting intraspecific variation in this region. All isolates amplified 540bp were recognized same fragment pattern as digested with *Alu* I, *Cfo* I, *Hinf* I, whereas consisted of two pattern as digested with *Hae* III. On the other hand, one isolate of 560bp product were detected unique fragment pattern. These results suggested that the isolates used in this study were belonging to *L. muscarium* and *L. longisporum* except for one isolate, which alone could not be belonged to both *Lecanicillium* spp. This result was associated with the previous study that was divided into three groups according to DNA polymorphisms of mitochondrial large subunit rDNA and Bt-2 region of β-tubulin gene, but not ITS, IGS, mitochondrial small subunit of rDNA and histone 4 region (Sugimoto *et al*, 2003).

Interactions between rhizoplane bacteria and a phytopathogenic Peronosporomycete *Aphanomyces cochlioides* in relation to the suppression of damping-off disease in sugar beet and spinach

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Zoospores play an important role in the infection of plant and animal hosts caused by *Perosporomycetes*. *Lysobacter* sp strain SB-K88, isolated from the root of sugar beet produced xanthobaccins and suppressed damping-off disease in sugar beet caused by *Pythium* species. Rhizosphere colonization of bacteria is thought to be crucial for their biocontrol effects. We investigated the modes of root colonization and antibiosis of SB-K88 and other bacterial strains isolated from the rhizoplane of spinach and sugar beet antagonizing a Peronosporomycete pathogen, *Aphanomyces cochlioides,* with respect to the suppression of damping-off disease in sugar beet and spinach. SB-K88 has huge long brush-like fimbriae on one pole of the sessile bacterial rod. To investigate the mode of colonization, seeds were inoculated with SB-K88 and sown in sterilized gellan-gum based gel in test tubes or soil in small pots. Scanning electron microscope (SEM) analysis of two weeks old seedlings revealed that i) SB-K88 densely colonized to the root and cotyledon surfaces in a perpendicular fashion using polar fimbriae; ii) a biofilm-like structure covered by root mucigel; iii) micro-colonies localized mainly at the junction between primary and secondary roots with bacterial numbers declining drastically from the top to down the root; and iv) root hairs of bacteria inoculated seedlings grown in gellan-gum were almost free from bacteria but they were shorter in size and more swollen than those of control. Seed treated with either SB-K88 or its cell free culture supernatant significantly suppressed damping-off disease in both sugar beet and spinach when seedlings were artificially infested with the zoospores of *A. cochlioides*. In addition to mycelial growth inhibition, zoospore of *A. cochlioides* was rendered immotile within 1 min of exposure to cell suspension or cell culture supernatant of the SB-K88, and subsequent lysis occurred within 30 min. Crude organic extract (MIC 10 μg/ml) of the culture supernatant or pure xanthobaccin A (MIC 0.01 μg/ml) also caused identical motility inhibition followed by lysis of zoospores in a dose dependent manner. Chemical fractionations of crude extract suggested that xanthobaccin-like antibiotics produced by the SB-K88 were primarily responsible for mycelial growth inhibition and lysis of zoospores. Bioassay revealed that very few zoospores approached the root tips inoculated with the SB-K88, and the motility of zoospore around the root tips was impeded. Rather than germinating, the zoospores were also partly lysed. Our observations provide the convincing evidence that SB-K88 exerts a direct inhibitory effect on *A. cochlioides* and suppresses damping-off disease in sugar beet and spinach through a combination of antibiosis and high root colonization. Similar phenomena were also observed in other rhizoplane bacteria. Most of them showed remarkable suppressive effects on *A. cochlioides* infection to sugar beet and spinach roots. The modes of colonization on host roots as well as growth inhibition of mycelia and zoospore lytic activity of bacterial secondary metabolites visualized by TEM and SEM will be presented. The results will be discussed in relation to the biorational control of soilborne Peronosporomycete phytopathogens.

Studies on efficacy and mode of action of rhizosphere bacteria against *Phytophthora* sps in strawberry

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After screening of several rhizosphere bacteria against the soilborne pathogens of Red core and Crown rot disease of strawberry *Phytophthora fragariae var.fragariae* and *Phytophthora cactorum* under in vitro conditions, three of the most active isolates which produced up to 63% of reduction in mycelium growth, such as *Raoultella terrigena* (G-584), *Bacillus amyloliquefaciens* (G-V1) and *Pseudomonas fluorescens* (2R1-7) were selected for further studies under *in vivo* conditions. In a greenhouse experiment two of the isolates, *Raoultella terrigena* (G-584) and *Bacillus amyloliquefaciens* (G-V1), were tested on their biocontrol effect against both *Phytophthora* diseases in comparison to the chemical fungicide Aliette. The experiment was carried out with artificial infested soil, using the highly susceptible strawberry variety ELSANTA. Root dip treatment with the bacterial antagonists produced a control effect on both fungal diseases between 52% to 64%, which was nearly comparable with Aliette. Moreover plant biomass was studied by measuring fresh and dry weight of shoots and roots of plants treated with antagonists and Aliette. Both parameters increased when compared to untreated control up to 38%. In a field experiment, mentioned above three isolates were tested against both *Phytophthora* diseases under artificial infested soil conditions. Root dip treatment with the bacterial antagonists produced a control effect on both fungal diseases up to 43%. Strongest activity was observed by *Pseudomonas fluorescens* on Red core 43% and Crown rot 36 % followed by *Raoultella terrigena* (Red core 31% and Crown rot 32%) and *Bacillus amyloliquefaciens* (Red core 34% and Crown rot 27%). In addition, isolation and identification of *Phytophthora* spp. from naturally infected soil from fields of different growers were undertaken, in order to confirm the natural infected soil status for field experiments. Isolates were identified at the genus level by using selective media, microscopic observation and plant disease detection kit (ALERT, Neogen corporation, USA).

In first studies on the mode of action of the antagonistic isolates, G-V1 from *Bacillus amyloliquefaciens* showed some indication of Cellulase and Glucanase enzyme production on different media; further investigations are underway to find out other possible mode of action against both *Phytophthora* spp. Further biocontrol studies are in progress on different locations of Germany under naturally infected soil conditions with strawberry farmers. Studies are made in cooperation with the firm E-Nema, Raisdorf with the aim to develop an antagonistic preparation against both *Phytophthora* diseases.

Evaluation of side effects of biocontrol agents on mites (tetranychid and predatory mites)

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Some biocontrol agents are currently used against plant pathogens on several crops. It is always assumed that they are safe to beneficial mites and they do not influence the spider mites *Tetranychus urticae*. Some species of *Phytoseideae* can feed on pollen and spores present on the leave surface. This could be a positive effect in periods in which the prey is not present at sufficient level. The aim of this research is to exclude any possible side effect of some commercial BCA, influence on the increase of parasite mites populations and evaluate if BCAs can be an alternative food for predatory mite.

Three commercial products based on *Trichoderma harzianum* T39 (Trichodex), *Bacillus subtilis* (Serenade) and *Ampelomyces quisqualis* (AQ10) and an experimental strain of *B. subtilis* were tested on *Amblyseius andersoni* female adults according to the guidelines of IOBC/WPRS. The effect on *T. urticae* was evaluated. BCAs have no side effects on predatory mites. Electrophorectical analyses of predatory mites were performed to evaluate their feeding activity on BCAs.

Screening and Identification of potential biocontrol agents against grapevine downy mildew considering an integrated control strategy of the disease

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Grapevine downy mildew agent, *Plasmopara viticola* (Berk *et* Curt) Berl et De Toni, is one of the most important grapevine pathogens, which can cause high losses if chemical treatments are not applied. Nowadays few biocontrol agents are successfully applied against several diseases. Due to its epidemiological characteristic (long lasting oospores, high production of sporangia, short time required for infection, and destructive effect of infections in some stages of the plant) the use of a single BCA is not sufficient to reach a satisfying control of the downy mildew of grape. An approach that integrates BCAs active against both the overwintering stage (oospores) and the sporangia could be more effective in managing the disease.

The aim of present work was to find and evaluate micro-organisms that can limit the development of *P. viticola* to be used as alternative to chemical fungicides with an integrated approach. 1746 micro-organisms (544 fungi, 894 bacteria and 308 yeasts) were isolated from vegetable material in untreated vineyards. The isolates were tested using four different methods to evaluate their capacity: to inhibit oospore germination and sporangia germination, to inhibit sporangiophore development and to globally reduce the incidence and/or severity of infections. During the first year 42,4 % of the tested organisms were effective in preventing germination of oospores overwintered in natural conditions, but only 9,3 % of them were effective in the second year. This raises again the issue of the importance of the environmental conditions in the activity of the potential BCAs. Among the tested organisms 7 were able to prevent sporangia germination, 10 to inhibit sporangia development and 47 are able to reduce infections in experimental conditions. The organisms effective against secondary infection s were tested on plants with artificial inoculation and they always reached a partial control of the disease. Micro-organisms, which can act as decomposers of grape leaves were also selected. Among the tested micro-organisms those, which has shown activity on different stages of the disease process, are selected for further studies in greenhouse and in field, for evaluating their potential use as grapevine downy mildew BCAs in an integrated approach in which both the overwintering and secondary inocula are targeted.

Control of powdery mildew on organic pepper

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Production of organic greenhouse vegetables in Israel which most of its production is for export to Europe (14,500 tons of organic vegetables, in 2002) is economically important, and is increasing during the recent years. Powdery mildew of pepper, caused by *Leveillula taurica*, is a disease affecting most commercial varieties. Disease symptoms include white powdery mycelial growth on the underside of the leaves, and chlorotic spots on the upper surface of the leaves, which turn to be necrotic. Affected leaves tend to drop off the plant, causing direct damage to the fruits, due to exposure to sunlight, in addition to the reduced photosynthesis capacity of the plant. In organic management sulphore-containing agents are used efficiently to control the disease. However, since sulfur agents may harm beneficial insects spread in the greenhouse to control pests, our main objective in this study was to search for alternative control means. Two experiments were carried out under full organic management, in protected walking tunnels at "Yair" research station, located in the southern part of Israel. Fiesta and 107 cultivars were used in 1999-2000 season, and cvs. Nibla and Parker in 2000-01. In the first experiment, sulphore agents either sprayed on the foliage or fumigated in the greenhouse, as expected, were the most efficient in controlling the disease. However, all the other tested treatments including water extract of cattle manure compost, Kaligrin (calcium bi-carbonate) and Rifol (fish oil) were better than the non-treated controls. Also in the second year, all treatments including Neemgard, water extract of grape marc compost, AQ10 (*Ampelomyces quisqualis*), Kaligrin and Rifol, significantly reduced disease incidence comparing to the non treated control, however, disease incidence in plots treated with sulphore was the lowest. Yields obtained in all treatments (especially in Neemgard) were higher than in the control, only with cv. Nibla.

Control of plant diseases by tea tree oil

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Tea tree oil is an essential oil steam distilled from the Australian plant *Melaleuca alternifolia*. Tea tree oil contains over 100 components, mostly monoterpenes, sesquiterpenes and their alcohols. This natural oil is an effective antiseptic, fungicide and bactericide, and has many safe and effective uses in the health and cosmetics industry. Its use against plant pathogens has not been investigated. Recently, two new formulations Timor and Timorex containing 50 and 66% tea tree oil, respectively, effective against broad spectrum of plant diseases in vegetables, herbs, grapevines and fruit trees, with no phytotoxicity to plant foliage were developed. *In vitro* tests showed that Timor and Timorex at 0.001-0.01% inhibited conidia germination and at 0.01-0.1% inhibited mycelial growth of various fungal pathogens belonging to powdery and downy mildews, and species of *Alternaria*, *Aspergillus*, *Stemphylium* and *Penicillium*. Growth chambers tests revealed that both compounds inhibited powdery mildews in grapevine (*Uncinula necator*) and cucurbits (*Spaerotheca fuliginea*). In greenhouses and open fields Timor at 1% mainly inhibited powdery mildews in cucurbits, pepper and grapevines. Pre-planting dipping of potato tubers in 0.5% solution of Timor inhibited *Rhizoctonia solani* infection by 71 and 99% in tubers of organic and conventional growth, respectively, compared to controls. Timorex at 1% controlled powdery mildews in herbs, carrot, mango, apple and nectarine, and was also effective in controlling cucumber downy mildew and potato early blight. Tea tree compounds do not harm natural enemies and can be used as replacement of sulfur and / or copper. Their mode of action is not clearly understood, but they act as protectant against wide range of fungi by inhibiting spore germination, mycelial growth and sporulation and by suppression of mildewed tissue.

Implementation of IPM strategies on greenhouse tomato on Oeste region of Portugal: case study

Sofia Rodrigues[1], Elisabete Figueiredo[2], Fernanda Amaro[1], Maria do Céu Godinho[1,3], Carla Miranda[4], António Mexia[1,2]

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Since the eighties protected vegetable crops were increased in Oeste region of Portugal, particularly tomato crop that represents 52% of the total area and 50% of the total national production. The aim of this study conducted in a modern greenhouse on rock wool and coconut fibre was to follow up the tomato crop under IPM. For this purpose observations were carried out weekly and the diseases and pest species were identified as well as the enthomophagous species present. The experimental work was designed in order to validate an IPM programme (risk assessment methodologies, decision tools) previously set for Oeste region. *Botrytis cinerea* Pers. and *Leveilulla taurica* (Lev.) were the most important air born diseases and *Fusarium oxysporum* f. sp. *lycopersici* was the problem that limited production. The key pests were whiteflies, *Trialeurodes vaporariorum* (Westwood) and fruitworm, *Helicoverpa armigera* (Hbn). Towards the end of the growing season *Aculops lycopersici* (Massee) were found attacking the crop and was the main pest problem constrained the crop cycle to finish. Mirids, *Coenosia attenuata* Stein and *Hyposoter didymator* Thumberg were the most common entomophagous species reported. The results of the IPM model validation are also presented concerning risk assessment evaluation, decision tools and control methods adopted.

Going underground: towards understanding the biological nature of soil suppressiveness to *Rhizoctonia solani* in sugar beet

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Rhizoctonia solani anastomosis group (AG) 2-2IIIB infects sugar beet seedlings and mature sugar beets. Decreases in yield, increased tare of beets and problems with storage and processing are the result of a severe infection. Cultural practices and use of (partial) resistant varieties may reduce damage, but additional practices are still needed to control rhizoctonia disease. Based on growers' experience and field experiments, soils can become suppressive to *R. solani*: *R. solani* may be present in the soil, but sugar beets are not diseased. Being able to evaluate soils for their level of suppressiveness may be an asset for disease control. A bioassay was developed to determine soil suppressiveness to *R. solani*. Soil samples taken from commercial sugar beet fields differed in their level of suppressiveness. Several mechanisms can be involved in soil suppressiveness. It is hypothesized that soil suppressiveness to *R. solani* in sugar beet has a biological nature. A strategy is considered to confirm the biological nature and also to determine whether entire microbial communities are responsible for suppression (general suppression) or specific microbial microorganisms (specific suppression). *R. solani* may be suppressed by antagonistic microorganisms (e.g. competition for nutrients and infection sites or hyperparasitism) or by toxic compounds released by these microorganisms (e.g. antibiotics, enzymes). PCR-DGGE and HPLC will be used to determine microbiological diversity and production of compounds in suppressive and conducive soils.

Disease management on organically grown cyclamen

Giovanni Minuto[1], **Andrea Minuto**[2], **Federico Tinivella**[2], **Maria Lodovica Gullino**[2], **Angelo Garibaldi**[2]

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The organic production is one of the most stimulating agricultural sector, both from the economic and scientific point of view. Consumer's interest is increasing, particularly in northern Europe, also in the field of organically grown ornamental plants. The main problem is to satisfy grower's and consumer's needs: i) on the market are not available young ornamental plants obtained using the rules of organic production, also because the potential market, at the moment, is very small; ii) products for disease control must be available and effective; iii) fertilizers must be designed especially for floriculture, as, for example, substrates that, possibly, could have a suppressive activity; iv) the quantity and the quality of productions must be comparable with that of the "conventional" ones; v) the pots should be made by using biodegradable materials, to compost them at the end of the cultivation time; and vi) any product must meet EU rules (REG. CE 2092/91). For these reasons, it is necessary not only to solve specific phytopathological or cultural problems, but it is also necessary to face production problems from a more general point of view, in order to indicate how to obtain a final product completely organically grown. This special approach was applied on cyclamen, important flower crop grown as potted plant. The results of three years of experimental trials on setting up biological control and cultivation strategies on cyclamen indicate that hard research is still necessary in order to reach applicable and sustainable solutions for the grower. Unfortunately, for organic ornamental production, fertilizers and biological control products are not sufficient to solve all cultural and phytopathological problems and organic production strategies set up especially for cyclamen are not available. Some antagonists, i.e. *Fusarium* spp., gave good control of *Fusarium oxysporum* f. sp. *cyclaminis* as copper sulphate reduced damages caused by *Erwinia carotovora* subsp. *carotovora*. Inadequate, for this flower plants, is the control of *Botrytis cinerea* obtained by applying *Trichoderma harzianum*. Also for this reason, it is very important to control and to regulate the climate under greenhouse and to adopt cultural techniques (i.e. plant density, farm hygiene, healthy propagative material) that can create unfavourable conditions for disease diffusion and incidence. The evaluation of the commercial quality of plants at the end of trials indicated that it is very difficult to obtain good production without fertilizers specifically designed for organic floriculture.

Glasshouse and field evaluation of the antagonistic rhizosphere bacteria *Bacillus subtilis* 5-127 and *Paenibacillus alvei* K-165 in controlling *Clavibacter michiganensis* subsp. *michiganensis* of tomatoes

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Bacterial canker is one of the most serious bacterial diseases of tomato. Our attempt was focused on controlling the soil phase of the disease with biological factors. Experiments were conducted both in a glasshouse and in the field. During 1999-2001 glasshouse experiments were carried out to induce systemic resistance in tomatoes against *Clavibacter michiganensis* subsp. *michiganensis (C.m.m.)* with rhizosphere bacteria belonging to *Pseudomonas* sp. (fluorescent pseudomonas isolates Pf-17 and Pf-34) and K-165 strain of *Paenibacillus alvei*.

The tomato hybrid NOA F1 was drenched at the stage of four fully expanded leaves. *Pseudomonas* sp. (fluorescent pseudomonas isolates Pf-17 and Pf-34) and K-165 were applied as soil drench (30 ml 10^7-10^9cfu / ml). Seven to ten days after the application the plants were inoculated with *Clavibacter michiganensis* subsp. *Michiganensis.* The inoculum was introduced by injection in the stem at a concentration of 10^8 cfu/ml and the wound was protected with Vaseline. The experiment was repeated three times and 20 plants per treatment were used. Symptom assessment revealed that K-165 was capable of significantly reducing disease symptoms down to 52.89 % compared to the 72.78% of untreated control, while Pf-17 (63.02%) and Pf-34 (65.0%) were rather ineffective.

Further field evaluation of several antagonistic bacteria was also attempted against *Clavibacter michiganensis* subsp. *michiganensis*. Rhizosphere *Bacillus subtilis* strain 5-127, *Paenibacillus alvei* strain K-165, and the fluorescent *Pseudomonas* sp. strain Pf-17 while the pathogenic strain *C.m.m.*4007 of the pathogen were used. Tomato stems of 2-3 cm infiltrated with bacterial inoculum were buried at the sites of plant transplanting of GARNET F1 tomato hybrid and *C.m.m* drenching with 30 ml10^8 cfu/ml /plant was followed. Two weeks later the plants were drenched for the second time. Ten and twenty day's later tomato plants were again drenched with antagonistic bacterial suspensions. The percentage of the dead plants was 5% in the untreated control and 0% with treatments. Further symptom assessment referring to percentage of the diseased leaves revealed that K-165 was capable of significantly reducing disease symptoms down to 18.8% compared to the 31.5%, of untreated control, while Pf-17 (24.65%) and 5-127 (29.8%) were less effective. However, determining fruit setting and the mean number of marketable size tomato fruits, showed that control plants carried only 3.55 fruits per plant, while K-165 treated plants 10.17, 5-127 treated plants 11, and Pf-17 treated plants 9.88. Generally it was shown that strain K-165 could be considered as the most promising biocontrol agent against bacterial canker of tomato.

Induction of systemic resistance against bacterial canker of tomato by BABA or the antagonistic bacterium *Paenibacillus alvei* K-165

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Induction of systemic resistance in tomato plants against *Clavibacter michiganensis* subsp. *michiganensis* was attempted by using a rhizosphere *Paenibacillus alvei* isolate (K-165) and the chemical factor BABA. The tomato hybrid NOA was drenched at the stage of two fully expanded leaves. *Paenibacillus alvei* K-165 strain was applied as soil drench (100 ml 10^8cfu / ml). Seven days after the application of K-165 the plants were inoculated with *Clavibacter michiganensis* subsp. *michiganensis* The inoculum was introduced by injection in the stem at a concentration of 10^6 cfu/ml and the wound was protected with Vaseline. The experiment was repeated three times and 15 plants per treatment were used. Symptom assessment revealed that K-165 was capable of reducing disease symptoms by 30 – 40 %, compared to the untreated control. Indeed statistical analysis demonstrated significant differences for the biocontrol agent in comparison with the untreated control. These data are in agreement with previous results proving that K-165 triggers induced systemic resistance against other vascular parasites.

In the second approach induction of systemic resistance was attempted by using the chemical β–aminobutyric acid (BABA) against *Clavibacter michiganensis* subsp. *michiganensis*. The tomato hybrid NOA was drenched at the stage of two fully expanded leaves with 60 ml water solution of BABA, at a concentration of 2 mg/ml. Seven days after the application of BABA the plants were inoculated with *Clavibacter michiganensis* subsp. *michiganensis* at a concentration of 10^6 cfu/ml. The inoculum was introduced by injection in the stem and the wound was also protected with Vaseline. The experiment was repeated three times and 15 plants per treatment were used. Symptom assessment revealed that BABA was capable of reducing disease symptoms by 50–60% compared to the untreated control. Indeed statistical analysis demonstrated significant differences for the chemical agent in comparison with the untreated control. Although plants developed stress symptoms following root drenching with BABA such as chlorosis, necrotic spots and wilting, they recovered later.

Evaluation methodologies and mode of action of epiphytic vine yeasts against *Botrytis cinerea* of grapes

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Several epiphytic yeasts, collected from various vineyard regions of Greece were evaluated for their potential in preventing development of *Botrytis* gray rot. The procedure involved wounding by piercing a hole of 2 mm diameter in the middle of mature berries and dipping them to yeast suspensions (10^7 cfu/ml) one day prior to *Botrytis cinerea* inoculation (10^5 conidia/ml). After five days of incubation (RH 100%, temp 22C, photoperiod 12h), it was demonstrated that the two most effective isolates belonging to *Aureobasidium pullulans* and *Metschnikowia pulcherrima* were able to significantly reduce the rotted area of berries by 78 and 58 % respectively, and restrict *B. cinerea* sporulation up to 94 and 74% respectively.

Preliminary data concerning mechanisms of action of the most effective strain of *A. pullulans,* designated as TW1, indicate that the main mechanisms involve antagonism for nutrients. It was also indicated that strain TW1 was able to induce systemic resistance against *B. cinerea* on berries while no chitinase activity was detected. Interestingly enough volatiles including phenyl ethyl alcohol and ethanol, produced from strain TW1, may be involved in its observed antagonistic activity against *B. cinerea.*

Development of a bio-inseticide based on a cold-active entomopathogenic nematode

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The larvae of *Otiorhynchus sulcatus* F. (Coleoptera, Curculionidae) feed on the root system of several plant species (mainly ornamentals and nursery stock). Biological control is a promising alternative approach to the chemical strategy, which has been accused to have a negative impact on the environment and on the insect resistance. It is very well known that entomopathogenic nematodes can effectively control the larvae of this pest, however the commercially available strains are only effective at temperatures above 12°C. On the other hand, the larvae of *O. sulcatus* develop also during winter and they feed even at temperatures as low as 5°C; for this reason nematodes active at these low temperatures could be very useful for the biocontrol of these insects.

Several nematode isolates found in soil collected in 1994 in the Italian Alps were screened under conditions as close as possible to the real environment in which the insect develops. Potted plants grown on peat, infected with larvae of *O. sulcatus* and treated with 0.5 x 10^6 nematodes/m^2 were used and results were evaluated between 14 to 21 days after treatment. The first screening has been done at 12°C, then 8°C and at the end, 5°C. At this last extreme temperature, only one of those isolates gave insect mortality between 80 to 90%. A commercial strain of *Steinernema feltiae* was always used as the reference material. The efficacy of this commercial strain was comparable to the cold active isolate described here, only when tested at 20°C, and it was far lower at extreme temperatures. The described isolate can be used for the control of *O. sulcatus* larvae within a wide range of temperatures from as low as 5°C to a maximum of 20°C. In field trials, the selected nematode demonstrated to be very effective at very low temperatures. By mean of RAPD-PCR analysis, it has been established that the nematode belongs to the species *Steinernema kraussei*. In an *in vivo* production test, the cold-active isolate showed an optimal growing temperature of 15°C. In liquid fermentation satisfactory yields were obtained also at 20°C.

Antibacterial activity of essential oils

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The control of bacterial diseases of plants, which may be destructive under certain environmental conditions, is a considerable problem in the agriculture practice because of the limited availability of bactericides and the ability of a large number of phytopathogenic bacteria to spread, even at long distances, by contaminated and/or infected seeds. Besides the antibiotic and copper compounds, which for some extent present undesirable attributes either for the human and animal health or for the environment, no other active principles are available for the bacterial plant disease control. Furthermore, antibiotics oxytetracycline and streptomycin, used on plants in USA, are actually forbidden in the agricultural practices in many Countries to avoid the possible selection of individuals resistant to the drugs and hence the possible horizontal genetic transfer to animal and human pathogens. In addition, also the use of copper compounds, because of their general toxicity and mainly for the impact on the environment, is on the way to be restricted and controlled in the European Union through rule n° 473/2002. Many studies have pointed out on the possibility to use the essential oils and/or their components in medical and plant pathology as well as in the food industry for the control of micro-organisms pathogenic to consumers and/or responsible for food spoilage. The search for natural substances possibly harmless for the consumers and for the environment and useful for the protection of crops parasites has become a foreground need. The request of reduction in the use of the pesticides in agriculture prompts the need for the development of alternative active compounds and/or methods for the control of plant bacterial diseases to be used in integrated crop management as well as in bio-organic agriculture. The objective of this study was to evaluate the antibacterial activity *in vitro* of essential oils toward bacteria responsible of diseases on plants and cultivated mushrooms.

Essential oils extracted from fruits of caraway, coriander, cumin and fennel, were assayed *in vitro* for antibacterial activity toward two laboratory and 29 phytopathogenic bacterial species as well as responsible of cultivated mushrooms diseases. Significant antibacterial activity was shown against Gram positive and Gram-negative bacteria belonging to *Clavibacter, Curtobacterium* and *Rhodococcus* and *Erwinia, Xanthomonas*, *Ralstonia* and *Agrobacterium* genera, respectively. A much-reduced effect was observed for the fennel oil.

The availability of new active principles such as essential oils or their components is of great interest. The essential oils may be useful bactericides for the control of plant bacterial diseases and, in particular, in a bio-organic agriculture. Of particular interest is the possibility to use the above substances for seeds treatment. The significant antibacterial activity of essential oils toward the bacterial pathogens of mushroom suggest the possibility to use the substances also on this crop. Of course, other studies are necessary to evaluate the toxicity of the above substances toward seeds and/or plants and to set the appropriate formulations useful for the purpose.

Combined use of insectpathogenic fungi and nematodes against the onion thrips, *Thrips tabaci* in the field

Kerstin Jung

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In the year 2003, field trials against *Thrips tabaci*, an important pest insect in onion, leek and chives, were conducted at four different sites with commercially available products based on insectpathogenic fungi and nematodes. The trials were performed according to the EPPO guideline PP 1/85(3) for the efficacy evaluation of insecticides "Thrips on Outdoor Crops". As a chemical standard Perfekthion® was used in onion. In leek, Spruzit® and Neudosan® were applied alternately. The biocontrol products used were Mycotal® (*Lecanicillium muscarium*, former *Verticillium lecanii*, Koppert NL), PreFeRal® (*Paecilomyces fumosoroseus*, Biobest BE), Naturalis L® (*Beauveria bassiana*, Intrachem IT), Nemaplus® (*Steinernema feltiae*, E-Nema DE) and Nemagreen® (*Heterorhabditis bacteriophora*, E-Nema DE). They were applied either alone or in a mixture using common spray equipment. A wetting agent was added. The nematodes were applied at a rate of $1x10^6$ infective juveniles/m^2. The dosage of the fungi was 1 kg (1.5 l in the case of Naturalis L®)/ha. The treatments started mid June and were repeated up to six times in weekly intervals.

Throughout the summer, *T. tabaci* was recorded only in medium numbers (mean of 30 specimen/plant max.) at all four sites. In two trials (onion, Mainz; chives, Heilbronn), no differences between treatments and the untreated control could be detected. In the third trial (onion, Schifferstadt), a significant reduction was recorded for the treatment 'PreFeRal+Nemaplus', both in the number of thrips/plant (2 compared to 6 in the control) and the frequency of infestation (38 % compared to 93 % in the control), one week after the final application (three times in total). Also in the fourth trial (leek, Bonn), the number of thrips/plant was lowest for the treatments 'fungi+nematode' (4 compared to 7 in the control, respectively) one week after the final application (six times in total). In this trial, yield was measured additionally, and it turned out, that the weight/plant registered for the treatments 'Nemaplus' and 'Mycotal+Nemaplus' was 20 % higher than in the control (425 and 412 g/plant compared to 345 g respectively).

All in all, these trials show that the use of insectpathogenic fungi and nematodes in the field is possible, confirming results of others collected in Great Britain and the Netherlands. In consideration of the extreme weather conditions in summer 2003 in Germany, and in comparison to the effect of the chemical standards, the results gained with the biocontrol agents are regarded as very promising. However, more experiments are necessary.

Meeting information

Meeting information

Meeting secretary
+39 0461 615239

Mobile phone meeting secretary (from 7 June to 13 June)
+39 335 8359262

Mobile phone Ilaria Pertot
+39 335 8359204

Accommodation

Trentino Holidays – Via Solteri, 78 – 38100 Trento – Italy; by fax n. +39 0461 825657
Contact person: Evita Bondì e-mail: evita@thol.it phone +39 0461 822554 or 822000

Banking facilities

There are many banks situated close to the Hotels in Trento, which offer currency exchange facilities. Banking hours are from 8.00 – 13.00 and from 14.30 – 15.30 Monday to Friday.

Automatic Teller Machines (ATMs) or cashflow machines are available throughout the city. There is one located on the ground floor of the Institute

Cassa Rurale di Mezzolombardo e S. Michele a/Adige
S. Michele a/A
piazza Chistè, 6 – 38010 S. Michele all'Adige
Tel: 0461 610160
Fax: 0461 650880
Hours: 8.05 - 13.05 / 14.35 - 15.35 Monday to Friday

Cassa Rurale di Trento
Trento city (near hotel Accademia and tourist office)
Via Balenzani, 4 - Trento
Tel: 0461 206201
Fax: 0461 206209
Hours: 08.05 - 15.45 Monday to Friday

Cassa Rurale di Trento
Trento city (near all the hotels)
Via Manci, 87 – Trento
Tel: 0461 206695
Fax: 0461 206699
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Catering during Meeting

Morning and afternoon coffees, lunches the dinners of 11 and 12 June and are included in the registration fee. Tickets for meals and coffee breaks will be provided at registration time. The bar of the Institute is open from 8.30 to 16.00.

Mobile phones

Mobile phones MUST be turned off once inside the lecture theatre.

Electricity

Italy uses European plugs (220 volt 50 HZ system with 3 pin flat plugs).

Email/internet access

A computer is located in the room D on the first floor of the Institute and is available during Meeting hours.

Facsimiles

Faxes can be received, please contact the Meeting Secretariat office on the first floor of the Institute.

Goods and services tax

Goods and services in Italy are subject to a 20% IVA (VAT). This is always included in the price.

Group picture

The meeting for the group picture will be at main entrance of the Institute, on Saturday 12 June at 13.50.

Medical services

First aid (Trentino Emergenza):
Call 118

Medical assistance (Guardia Medica):

Trento, Via Malta, 4 (night and holidays)
Tel +39 0461 915809

San Michele all'Adige (night and holidays):
Tel. +39 0461 61 11 11

Hospitals in Trento:

Ospedale S.Chiara, Largo Medaglie d'Oro, tel +39 0461 903111
Ospedale San Camillo, Via Giovannelli, 19, tel +39 0461 216111

Parking

A car park is available at the Institute right to the entrance.

Pharmacies

The nearest pharmacy to the Meeting venue is:

Farmacia Tomazzolli dr. Marcella
Via Biasi, 8
38010 S. Michele all'Adige
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Opening hours: 8.30 – 12.00 /15.00 – 19.00 Monday to Saturday
The pharmacy will be closed on Tuesday afternoon and on Saturday afternoon

Pharmacies in Trento, near the hotels:

Farmacia alla Madonna
Via Giannantonio Manci 42
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38100 Trento
tel. +39 0461 827944
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8.30 – 22.00 (12 and 13 June 04)

Phones

Public phones are located in the basement of the Institute. A phone card or coins are needed to make a local or international call. Phone cards can be purchased at the registration desk.

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The closest post office to the Institute is:

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via Francesco Biasi,7
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Poster

Posters must be hanged on Thursday 10 June (from 8.30, before the opening session). Manning of the posters by the presenting authors is expected during the poster sessions.

Registration and information desk

The Meeting Registration and Information Desk is located in the first floor of the Institute (B).
Opening Hours: 8.00-18.00

Shopping

Shopping hours in Trento are usually: 9.00 – 12.00, 15.00 – 19.00 Monday to Saturday. The shops are closed on Monday morning and on Sunday.

Smoking

Smoking is not permitted in all the buildings of the Institute.

Speaker preparation room

The Speaker preparation room is located on the first floor of the Institute (see map). The room has a computer with MS PowerPoint. Staff at the computer help desk located outside the speaker room, are available to provide assistance.

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Azienda di Promozione Turistica di Trento (**Trento Tourist Board**)
via Manci 2 - Trento
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Radio taxi Trento
via Alcide Degasperi 27
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www.taxitrento.it

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Lufthansa Tel. 02 80663025
Hours: every day from 8.00 - 21.00

Austrian Airlines Tel. 02 80663095
Hours: every day from 8.30 – 21.00

Air Dolomiti	Tel. 800 013366
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British Airways	Tel. 199.712266
KLM	Tel. 199.414.199
Iberia	Tel. 8488.26236
Air Portugal	Tel. 848.888.910

Useful phone numbers

Carabinieri: Via Barbacovi, 24, tel +39 0461 983113 (112)

Police (Questura - Polizia di Stato): Piazza Mostra, 3, tel 39 0461 899511 (113)

Local police (Polizia Municipale): tel +39 0461 916111

Train station (Trento): Piazza Dante, tel +39 0461 891411

Train station (Trento-Male): Via Secondo da Trento, tel +39 0461 238350

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G Bar
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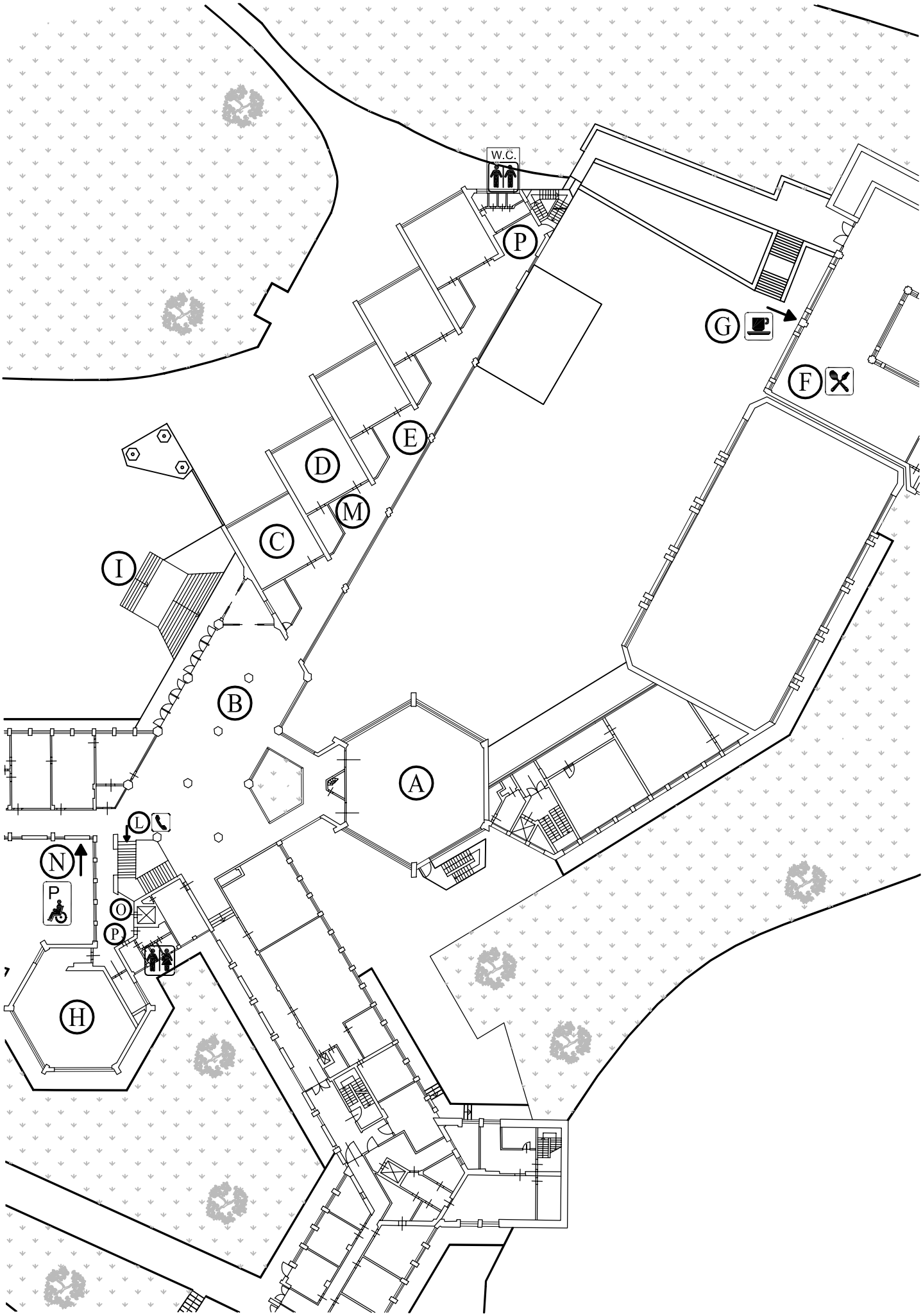
M ATM
N Access for disables
O Elevator
P Toilettes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