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Development of a granulovirus based insecticide to control the potato tuber moths,

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Analysis of several Colombian *Phthorimaea operculella* granulovirus isolated from

***Tecia solanivora*: Detection of a new variable region in the PhopGV genome**

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- Acaricidal activity of *Bacillus thuringiensis* toxins against mite pests
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- The efficiency of some biological insecticides against the Potato Tuber Moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) in organic potatoes in Cyprus
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- Sublethal effect of Bt-maize in semi-artificial diet on European corn borer larvae, *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera, Crambidae)
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- Abstract:** A laboratory study was conducted to determine the effect of Bt maize (Cry1Ab, event MON 810) incorporated in a standard semi-artificial diet on larval development and survival of different European strains of *Ostrinia nubilalis*. The strains originated from 2 different localities in Serbia and Slovakia, 1 locality in Germany, Romania and Austria. A simple method was designed to evaluate Bt maize tissue consuming on development of *O. nubilalis* larvae in laboratory conditions. The larvae were reared on semi-artificial diet enriched with powder of Bt maize leaves. The bioassay demonstrated slower development of larvae on Bt diet and higher mortality of larvae when compared to individuals reared on non-Bt diet. The range of variation in larval development and survival was great and indicated a natural variation in Bt susceptibility among geographically distinct populations of *O. nubilalis*.
- Binding and pore formation of Cyt1Aa and Cry11Aa toxins of *Bacillus thuringiensis israelensis* to brush border membrane vesicles of *Tipula paludosa* (Diptera: Nematocera)
J. Oestergaard, R.-U. Ehlers, A. C. Martínez-Ramírez, M. D. Real (Abstract only) 131
- Susceptibility of *Tipula paludosa* against *Bacillus thuringiensis israelensis*, *Steinernema carpocapsae* and *Steinernema feltiae*
O. Strauch, J. Oestergaard, R.-U. Ehlers 132
- Abstract:** *Tipula paludosa* (Diptera: Nematocera) is the major insect pest in grassland in Northwest Europe and is an invasive species in North America. Oviposition occurs during late August and first instars hatch from September until mid-October. Laboratory and field trials were conducted to assess the control potential of entomopathogenic nematodes (EPN) (*Steinernema carpocapsae* and *S. feltiae*) and *Bacillus thuringiensis* subsp. *israelensis* (Bti) against *T. paludosa*. Results indicate that the early instars of the insect are most susceptible to nematodes and Bti. In the field the neonates prevail when temperatures tend to drop below 10°C. *S. carpocapsae*, reaching > 80% control, is more effective against young stages of *T. paludosa* than *S. feltiae* (< 50%), but the potential of *S. carpocapsae* is limited by temperatures below 12°C. Mortality of *T. paludosa* caused by Bti was not affected by temperature. Even at 4°C insects were affected but the lethal time increased with decreasing temperatures. Synergistic effects of Bti and EPN against *T. paludosa* were observed in 3 out of 10 combinations in laboratory assays but not in a field trial. The potential of *S. carpocapsae* was demonstrated in field trials against early instars in October reaching an efficacy of >80% with 0.5 million nematodes per m² at soil temperatures ranging between 3° and 18°C. Against early instars in autumn between 74 and 83% control was achieved with 13 kg/ha Bti of 5,700 International Toxic Units (ITUs) and 20 kg/ha of 3,000 ITUs. A wheat bran granular formulation with Bti

was superior in efficacy than the sprayed product against L3 stages in an application at the end of November. 10 kg/ha Bti had no effect when it was sprayed, whereas the same amount of Bti applied with the granular formulation had a significant efficacy of 68 % (Abbott corrected). The results indicate that spray applications of Bti and nematodes will only be successful and economically feasible during the occurrence of early instars. At temperatures above 12°C entomopathogenic nematodes are an effective alternative to Bti against the first instars of *T. paludosa*, although Bti products might be cheaper.

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| Cellular effects of the <i>Bacillus sphaericus</i> Binary toxin on MDCK cells expressing its receptor O. Opota, N. Gauthier, A. Doye, P. Gounon, C. Berry, E. Lemichez, D. Pauron (Abstract only)..... | 136 |
| New molecular phylogeny of <i>Photorhabdus</i> and <i>Xenorhabdus</i> based on a multigene approach A. Paule, S. Pagès, C. Laroui, S. P. Stock, P. Tailliez (Abstract only)..... | 137 |
| Identification and typing of <i>Photorhabdus</i> isolates from entomopathogenic nematodes in Hungarian soils T. Tóth, T. Lakatos, Z. Kaskötő | 138 |
| Abstract: <i>Photorhabdus</i> strains from entomopathogenic nematodes isolated from Hungarian soils were characterized by morphological, physiological and genetic properties to survey the diversity of bacterial symbionts of <i>Heterorhabditis</i> species of commercial importance. Entomopathogenic bacteria (EPB) were isolated from 245 entomopathogenic nematode strains originated from different part of Hungary. There were 156 <i>Photorhabdus</i> and 77 <i>Xenorhabdus</i> from the successfully cultured 233 EPB isolates. 65 <i>Photorhabdus</i> isolates representing the whole collection from the point of view of geographical and nematode host distribution were analysed. Primary form bacterial cells selected on NBTa indicator plates were used to determine the morphological traits and to perform physiological tests using Biolog GN microplates and API20E strips. Cytotoxic and antibacterial properties of cellfree culture broth were measured against <i>Drosophila melanogaster</i> S2 and <i>Spodoptera frugiperda</i> Sf9 cell lines or <i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i> bacteria, respectively. Morphologically and physiologically homogenous groups of <i>Photorhabdus</i> isolates were characterized by partial sequencing of the <i>gyrB</i> subunit gene. High physiological and morphological diversity was found among the <i>Photorhabdus</i> isolates and all of physiological and morphological bacteria types could be isolated both from <i>Heterorhabditis megidis</i> and <i>H. downesi</i> . The possible role of this diversity is discussed. | |
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| Abstract: <i>Photorhabdus</i> and <i>Xenorhabdus</i> produce metabolites which are active against bacteria and fungi. Substances with nematocidal activity have also been detected in fermentation broths of both bacteria genera. Strains of <i>Photorhabdus</i> and <i>Xenorhabdus</i> able to synthesize proteins with insecticidal properties have been selected. Since the encouraging results obtained by the use of these active metabolites and the recognition of their potential application in the medical and agricultural fields, several patents have been filed for the use of strains and their metabolites. Most of the patent applications are related to their insecticidal toxins and corresponding genes. In addition, there are also intellectual property applications related to innovative formulations useful to increase the activity of <i>Photorhabdus</i> insecticidal strains for field application. The importance of patent applications related to the agricultural field will be presented and their impact on biological control practice will be discussed. | |
| Exploiting the <i>Photorhabdus</i> genome R. ffrench-Constant, A. Dowling, M. Hares, J. Parkhill, N. Waterfield (Abstract only)..... | 150 |

Photorhabdus and Xenorhabdus: potent secondary metabolite producers

A. O. Brachmann, G. Schwär, H. B. Bode 151

Abstract: The entomopathogenic bacteria *Photorhabdus* and *Xenorhabdus* are sources of many secondary metabolites and over the last 25 years an increasing number of compounds could be isolated. Many of these compounds show biological activities and have an antibacterial, antifungal, antinematodal or cytotoxic effect. Moreover there are a few hints that secondary metabolites are also required for the interaction between bacteria and nematodes. The recently isolated xenofuranones and anthraquinones are examples of the nearly inexhaustible arsenal of secondary metabolites from these organisms.

New aspects on *Xenorhabdus* antibiotic research

A. Fodor, S. Forst, L. Haynes, M. Hevesi, J. Hogan, M. G. Klein, A. Máthe-Fodor, E. Stackebrandt, A. Szentirmai, F. Sztaricskai, T. Érsek, M. Zeller 157

Abstract: Antibiotics from bacterial symbionts of entomopathogenic nematodes may be used to control microbial pathogens of agronomic and medical importance. As a part of a systematic search for potent antibiotics, the taxonomic placement of 4 *Xenorhabdus* strains was determined on the basis of 16S rRNA gene sequences and RiboPrint patterns. They are *Xenorhabdus budapestensis*, *Xenorhabdus ehlersii*, *Xenorhabdus innexi*, and *Xenorhabdus szentirmaii*. Strong antimicrobial activity from *X. budapestensis*, and *X. szentirmaii* strains were demonstrated on Gram-positive and Gram-negative mastitis bacterial isolates in repeated tests and on *Erwinia amylovora*, the causal agent of fire blight; as well as on *Phytophthora nicotianae*. The cell-free media was also effective in repeated phytotron tests. Antibiotics from *X. budapestensis* have high thermo-stability, and the effects are cytotoxic. With mastitis pathogens, *Klebsiella pneumoniae* was the least susceptible, and *Staphylococcus aureus* showed the highest sensitivity to each *Xenorhabdus* strain tested. Nematophin, a previously reported antibiotic from *X. nematophila* was not among the compounds produced by *X. budapestensis*. The synthetic racemic nematophin proved ineffective against any bacteria tested. Xenofuranones A and B have been isolated from cultures of *X. szentirmaii*. Their structures were elucidated by NMR and mass spectroscopy. The structure of the water-insoluble, coloured, exocrystal produced by *X. szentirmaii* was determined as an antibiotic pigment (iodinin), when in solution, the iodinin is probably complexed to a polysaccharide, that can be formed from mannitol or adonitol, but not from D-glucose, D-fructose or glycerol.

Functional genomics of *Photorhabdus asymbiotica* - rapid virulence annotation (RVA) of pathogen genomes using invertebrate models

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Photorhabdus spp. and *Xenorhabdus* spp: Only feed for mass production of nematodes?

A. Peters 166

Abstract: A huge proportion in the nematode taxon Secernentea rely on bacteria as a food source. Many species within the Rhabditida, like *Caenorhabditis elegans*, are opportunistically feeding on several species of bacteria which live on decaying organic matter. The slug-parasitic nematode *Phasmarhabditis hermaphrodita* can be found in associations with various bacteria, while only few associations support good nematode growth and pathogenicity against slugs (Wilson *et al.*, 1995). The genera *Steinernema* and *Heterorhabditis*, however, have entered into a symbiotic association with specific bacterial symbionts. The symbiosis is obligating for the nematode but not for the bacteria, which will easily grow *in vitro* without the nematode symbiont. This unilateral nutritional symbiosis is the key to the success of these nematodes as biocontrol agents since it permits the mass production of the bacteria on cheap artificial media and at the same time ensures the formation of rather specific insecticidal nematodes. Besides the nutritional function of the symbiotic bacteria, they play an important role in modulating nematode development in the insect or in the mass production. Experience from mass production of *Steinernema feltiae*, *S. carpocapsae* and *H. bacteriophora* indicate that the conditions of the bacteria preculture affect the modulation of the nematodes' development by the bacteria.

Fungi

Assessing winter survival of *Pandora neoaphidis* in soil applying bioassay and molecular approaches

A. Fournier, F. Widmer, S. Keller, J. Enkerli..... 173

Abstract: *Pandora neoaphidis* (Entomophthorales) is one of the most important fungal pathogens of aphids and it has a great potential for use in biocontrol. As cultivation of *P. neoaphidis* is difficult, conservation biocontrol strategies are favoured. However, little is known on overwintering strategies of this fungus. It is hypothesized that natural areas may play an important role for survival and that undisturbed soil may serve as inoculum source for new populations in spring. To test these hypotheses, we have developed a cultivation-independent PCR-based diagnostic tool that allowed for detection of *P. neoaphidis* DNA in top soil samples collected during winter from a nettle field harboring infected aphids in fall. Results suggested an overwintering stage of *P. neoaphidis* in top soil layers. The PCR-based method, however, does not provide information on viability or virulence of detected *P. neoaphidis* material. Therefore, a field study was initiated in summer 2006 which will last until spring 2007. It aims at investigating winter survival of *P. neoaphidis* in top soil layers and to test whether *P. neoaphidis* material detected with the molecular tool represents infectious fungal material. For this purpose, molecular analyses of soil samples are accompanied with a bioassay in which aphids are placed on soil samples and *P. neoaphidis* infection is monitored, and recorded as aphid mortality. The experimental layout consists of eight replicated caged plots (0.16 m²) for each of four different treatments: 1) *paf* plots: lucerne plants inoculated with pea aphids and the fungus *P. neoaphidis*; 2) *pa* plots: lucerne plants with aphids but without artificial *P. neoaphidis* inoculation; 3) *p* plots: lucerne plants without aphids and *P. neoaphidis*; and 4) *bs* plots: bare soil, which was covered with a weed barrier fabric. Soil samples were collected at four different time points in 2006. To date, bioassays and molecular analyses were carried out on soil samples from *paf* plots. Our preliminary results indicate a good correlation between bioassay data and PCR-based data, and suggest a decrease of *P. neoaphidis* inoculum in soil after winter begins.

Partial purification and characterization of an insecticidal and antifeedant protein produced by the entomopathogenic fungus *Metarhizium anisopliae*

A. Ortiz-Urquiza, I. Garrido-Jurado, C. Santiago-Álvarez, E. Quesada-Moraga 178

Abstract: Entomopathogenic fungi (EF) produce some macromolecular compounds that may be pathogenicity determinants and an unexplored source of new insecticidal compounds of natural origin. In our screening approach to evaluate production of insecticidal proteins among our collection of autochthonous strains of EF, we found one *M. anisopliae* isolate that secretes a protein with insecticidal and antifeedant properties. The oral toxicity of the crude protein soluble extract (CPSE) from the shake culture of this isolate in Adamek's liquid medium was tested in a diet test against adults of *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) and with alfalfa leaf disks against 2nd instar *Spodoptera littoralis* (Lepidoptera; Noctuidae). For both species, mortality caused by the CPSE progressively increased as a function of protein concentration and exposure time. The insecticidal activity of the CPSE was significantly reduced after exposure to protease treatments and its showed high thermostability. The toxic protein has been completely purified by liquid chromatography.

Factors important for the use of *Neozygites floridana* in biological control of the two-spotted spider mite, *Tetranychus urticae*

I. Klingen, K. Westrum, S. S. Nilsen, N. Trandem, G. Wærsted (Abstract only)..... 186

Supplementary data to the biology and taxonomy of fungous strains from mites and insects

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Effects of *Beauveria bassiana* on *Ips sexdentatus* and on *Thanasimus formicarius*

B. M. Steinwender, R. Wegensteiner (Abstract only)..... 188

Horizontal transmission of entomopathogenic fungi between sexes of the Mediterranean fruit fly *Ceratitis capitata* Wied. (Diptera; Tephritidae)

E. Quesada-Moraga, I. Martín-Carballo, C. Santiago-Álvarez (Abstract only)..... 189

- Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. against *Ips amitinus* (Coleoptera, Curculionidae)
R. Wegensteiner, J. Weiser (Abstract only) 190
- Field application of entomopathogenic fungi against *Rhagoletis cerasi*
C. Daniel, S. Keller, E. Wyss 191
- Abstract:** Two myco-insecticides, Naturalis-L (*Beauveria bassiana*) and PreFeRal® WG (*Paecilomyces fumosoroseus*), were applied against adult *R. cerasi* Loew (Diptera: Tephritidae) in two orchards in north-western Switzerland in summer 2006. Both products were applied at a concentration of 5.75x10⁴ CFU/ml. With four applications at seven day intervals the whole flight period of *R. cerasi* was fully covered. During this period the relative humidity averaged 66.3% (5th June – 6th July), the temperature averaged 20.5°C. Under these conditions only Naturalis-L significantly reduced the number of damaged fruit (efficacy: 69-74%), whereas damaged fruits were not significantly reduced with PreFeRal (efficacy: 27%). For *B. bassiana* living fungal propagules were still detectable seven days after application, while the fungal propagules of *P. fumosoroseus* remained only three days. A control of *R. cerasi* with myco-insecticides seems possible under field conditions. However, application regime still has to be improved.
- Natural occurrence of entomopathogenic fungi infecting the Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera, Curculionidae) in Southern Italy
E. Tarasco, F. Porcelli, M. Poliseo, E. Quesada Moraga, C. Santiago Álvarez, O. Triggiani 195
- Abstract:** Surveys for natural enemies of the Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera, Curculionidae) were conducted in several urban areas and nurseries of Southern Italy in 2006. More than 350 larvae, pupae and adults of the weevil collected from 25 plants were inspected for natural mortality. Entomopathogenic fungi were found to be a major factor regulating *R. ferrugineus* populations, with a natural infected prevalence around 20%. Inside the palm trees presenting infected insects, about 90% of the individuals were found to be diseased. Two species of entomopathogenic fungi were found, *Beauveria bassiana* (Bals.) Vuill., the most common species and *Metarhizium anisopliae* (Metch.) Sorok. Interestingly, *Paecilomyces* sp. was also found to infect phoretic mites on Red Palm Weevil.
- Studies on ecological and physiological host range of entomopathogenic Hyphomycetes
R. Seskena, V. Petrova, M. Jankevica, L. Jankevica 198
- Abstract:** Researches on entomopathogenic fungi of agricultural pests were carried out in the Institute of Biology, University of Latvia. Occurrence of entomopathogenic Hyphomycetes and their host range were studied. Information on identified and isolated entomopathogenic fungi and their ecological hosts recorded in Latvia since 1957 was summarised. For determination of ecological host insects with the symptoms of mycoses (reduced movement, changes of color, cuticle covered with fungal mycelia or conidia) were collected. Common insect pathogenic Hyphomycetes recorded in Latvia are *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces farinosus*. Observations showed that *B. bassiana* had a broad host range. 19 hosts of *B. bassiana* were recorded. Fungus *B. bassiana* was associated with different orders of insects and mites: Coleoptera, Lepidoptera, Hymenoptera, Hemiptera, Diptera, and Tetranychidae. Laboratory tests showed that *B. bassiana* isolate are not specific to aphids. *Aspergillus flavus* were associated with pest orders: Diptera, Aphididae, Tetranychidae, and Phytoseiidae.
- Sampling and occurrence of entomopathogenic fungi in soil from *Cameraria ohridella* (Lepidoptera: Gracillariidae) habitats
E. Prenerova, R. Zemek, F. Weyda, L. Volter (Abstract only) 204

Dissemination strategies of the entomopathogenic fungus *Lecanicillium muscarium*
Zare, Gams 2000 in the host population of *Frankliniella occidentalis* Pergande
1895

S. Lerche, H. Sermann, C. Büttner..... 205

Abstract: The fungus *Lecanicillium muscarium* syn. *Verticillium lecanii* can be used to control populations of the western flower thrips *F. occidentalis*. In laboratory, semi-field and greenhouse trials, the infection and mortality of this host population were recorded (Hetsch 2004; Meyer 2007). At the end of the disease process, the fungus grew and sporulated very well on the cadavers of *F. occidentalis*. These sporulating cadavers were an effective inoculum source and therefore a centre of infection for the host population (Lerche 2005). Now, trials were conducted to describe the possibilities of fungal dissemination and the efficiency in this host-pathogen-relationship. The results show different possibilities of pathogen dispersal. One of the most important ways of dissemination is by the behaviour and movement of the hosts. The dispersal was horizontally within the population, in and between the generations. The second way of pathogen dispersal by the host was the contamination of the insects's habitat. This included the growth of the fungus after death of the host, the sporulation of infectious stages on casted off exuvia during molting and by the loss of spores during the movement of the hosts. The physical agent running water also dispersed the pathogen efficient within the host habitat. The results were discussed.

Preliminary investigations on the occurrence of arthropod fungal pathogens in Austria

C. Tkaczuk, S. Balazy, R. Wegensteiner..... 209

Abstract: The aim of this investigation was to improve our knowledge of fungal pathogens of insects and mites in Austria and to compare their distribution and density in the soil from different habitats. Twelve entomophthoralean species from different insects and one species from mites were identified. Especially interesting is the first record to Austria of *Erynia ovispora*, *Entomophthora israelensis*, *Pandora echinospora*, *Pandora vomitoriae*, *Zoophthora opomyzae*, *Zoophthora patchii* and *Neozygites floridana*, but also two Hyphomycetes species – *Hirsutella kirchneri* and *H. thompsonii* – on eriophyid mites. Three fungal species: *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* were isolated by means of selective medium method from the soil of different habitats in Austria. The total CFU density of entomopathogenic fungi was 4-times higher in the soil from field afforestations than adjacent conventionally managed arable fields. CFU of *B. bassiana* were the most numerous in the soil from all investigated forest sites.

Efficacy of entomopathogenic fungi in control of different stages of western flower thrips (*Frankliniella occidentalis* Pergande)

Z.A. Fiedler, D. Sosnowska (Abstract only)..... 214

The potential of *Beauveria brongniartii* and botanical insecticide to control *Otiorynchus sulcatus* larvae in potted plants

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Efficacy of a *Beauveria bassiana* (Vuillemin) strain against the Castniid palm borer

Paysandisia archon (Burmeister, 1880) under laboratory and natural conditions

S. Besse-Millet, A. Bonhomme, K. Panchaud 216

Abstract: *Paysandisia archon* (Lepidoptera, Castniidae) is native in Argentina and has become a severe palm trees pest. It was first detected on the French Mediterranean coast in 1999 and since declared as a quarantine pest. Unfortunately, no chemical control is efficient because of the insect life cycle and the palms biology. Thanks to the cooperation between two French companies, i.e. Vegetech and NPP (Natural Plant Protection, a subsidiary of Arysta), and the support from the city of Hyères-les-Palmiers, a strain of the entomopathogenic fungus *Beauveria bassiana* has been identified as pathogenic. This strain designated Bb147 is the active ingredient of Ostrinil®, a biocontrol agent registered in France for the control of the European Corn Borer (ECB). It has been tested under laboratory and natural conditions. In the lab, eggs and larvae have been inoculated with a spores solution resulting in a 24 % survival in the egg experiment and 100% mortality 14 days after the inoculation in the larval experiment. A very good efficacy of Bb147 has been recorded both on 1 to 19 days-old larvae of *Paysandisia*

archon. The lethal time 50 is less than 6 days. In a second experiment, a dose response has been observed, still with high larval mortality. A field trial has been carried out on young palms under artificial infestations of *P. archon* neonate larvae in insect-proof cages. Bb 147 causes both, larval mortality and a growth delay in surviving larvae.

Efficacy of entomopathogenic fungi against larvae of the horse chestnut leafminer

Cameraria ohridella Deschka & Dimic, 1986 (Lepidoptera: Gracillariidae)

M. Kalmus, H. Sermann, S. Lerche, C. Büttner..... 220

Abstract: Pathogenicity of *Lecanicillium muscarium* strain V24 (Zare & Gams, 2001) and *Paecilomyces fumosoroseus* strain P6 against larvae of the horse-chestnut leafminer *Cameraria ohridella* within mines was investigated under laboratory conditions. Aqueous suspensions of 1×10^7 conidia/ml were sprayed on eggs, deposited on leaves of seedlings with a manual sprayer. *L. muscarium* and *P. fumosoroseus* caused mortality of 98 and 96% on larvae hatching from contaminated eggs. Mortality of the untreated control was significantly lower (12%). In both fungal treatments only 4% of all larvae pupated successfully. All cadavers from the treatments were moulded. In some cases the mycelium grew out of the mines. Additionally, dimensions of the mines were determined within 21 days past application.

Efficiency of the entomopathogenic fungus *Lecanicillium muscarium* on hibernating pupae of *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera, Gracillariidae)

D. Richter, H. Sermann, C. Jantsch, B. Jäckel, C. Büttner..... 223

Abstract: Pathogenicity of strains of *Lecanicillium muscarium*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae*, *Beauveria bassiana* was investigated under laboratory conditions on naked pupae and pupae in their pupal cells and in a semi field trial. Aqueous suspensions of 2×10^7 conidia/ml were sprayed in the laboratory with potter towers on the pupae and pupal cells. In the semi field trial $2,2 \times 10^8$ conidia/ml were sprayed with a hand applicator on the leaves on soil. The naked pupae are susceptible against entomopathogenic fungi. Mortality of naked pupae occurred earlier in *Lecanicillium* and *Paecilomyces* than in *Metarhizium* and *Beauveria*. In the pupal cells fewer pupae died but these pupae were infected by the fungi. *L. muscarium* was able to infect 56% of the pupae. In the semi field trial the fungus infected pupae of *C. ohridella* in their pupal cells and passed into the next stage of adult. Together the fungus has reduced the population by approximately 60%.

Susceptibility of *Rhagoletis cerasi* to entomopathogenic fungi

C. Daniel, S. Keller, E. Wyss..... 228

Abstract: The effects of six fungus strains (Deuteromycotina: Hyphomycetes) on the mortality of different life stages of the European cherry fruit fly, *Rhagoletis cerasi* Loew (Diptera: Tephritidae), were assessed in a series of laboratory experiments. All fungus strains were able to cause mycosis to larvae and adults of *R. cerasi*. However, virulence varied considerably among the strains. Effects on L3-larvae were negligible. In contrary, adults were highly susceptible to fungus infection. *Beauveria bassiana* and *Paecilomyces fumosoroseus* caused 90 to 100% mortality and had a strong influence on fecundity. *Metarhizium anisopliae* also showed reliable effects. The pathogenicity of *Paecilomyces farinosus* was low. Higher conidia concentrations lead to higher mortality, whereas *B. bassiana* was most efficient at low concentrations. Young flies showed lower mortality rates than older flies but, sub-lethal effects on eclosion rate off eggs were greater in young than in older flies.

Influence of plant species and pests on the development of pathogenic fungus

Paecilomyces lilacinus in soil conditions

D. Sosnowska (Abstract only)..... 234

Exposure of greenhouse workers to fungal biocontrol agents in vegetable production

V. M. Hansen, A. M. Madsen, N. V. Meyling, J. Eilenberg..... 235

Abstract: In workplaces such as greenhouses, fungal spores are introduced into the environment to control plant pests and plant pathogens. Exposure of the airways to bioaerosols can cause irritation and respiratory symptoms including asthma. So far the exposure of growers to inhalable fungal spores is not well investigated and we do not have the data needed to assess the effect of adding additional spores, for example from microbial biocontrol products, to the

working environment. In this newly initiated project, we aim to monitor the levels of naturally occurring bioaerosols as well as introduced organisms in greenhouses and open fields. We use GSP samplers for air sampling and quantify microorganisms in the collected material with a modified CAMNEA method. We will also quantify fungal biochemicals in the samples. Here, we present and discuss the preliminary results from the first working environments visited.

The impact of soil treatment on soil mycobiota

M. Kirchmair, S. Neuhauser, L. Huber, H. Strasser..... 239

Abstract: The changes in the composition of cultivable soil fungi after soil treatment with Agro Biosol® – an organic fertiliser based on fungal biomass – and the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*, used to control soil dwelling insect pests, were assessed by palting soil suspensions onto potato dextrose agar dishes. After one week incubation fungal colonies were counted and determined to genus level. For statistical analyses the twenty most common genera were pooled to six groups according their ecological characteristics: (i) saprobes of poor soils (*Acremonium* spp., *Aspergillus* spp., *Mortierella* spp., *Penicillium* spp), (ii) litter decomposers (*Alternaria* spp., *Cladosporium* spp, *Stachybotrys* spp., *Phoma* spp., *Ulocladium* spp.), (iii) Mucorales (Zygomycetes) as indicators of high organic fractions (*Absidia* spp., *Cunninghamella* spp., *Mucor* spp., *Rhizopus* spp.), (iv) mycoparasites (*Gliocladium* spp., *Trichoderma* spp.), (v) entomopathogens (*Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp.), and (vi) phytopathogens (*Cylindrocarpon* spp., *Fusarium* spp.). A positive influence of Agro Biosol® on mycoparasitic fungi could be observed by a canonical correspondence analysis. No influence on saprobes and plant pathogens could be found. The influence of *M. anisopliae* on soil fungi was mainly restricted to entomopathogens: Their density in soil increased (as desired) whereas the abundances of the other ecological groups were hardly affected. The method is sensitive for the assessment of fungal communities in soil and will be used in future studies to estimate the influence of different soil management strategies on soil fungi.

Antagonists of the spruce bark beetle *Ips typographus* L. (Coleoptera: Scolytidae) of German and Georgian populations

M. Burjanadze, J. C. Moser, G. Zimmermann, R. G. Kleespies 245

Abstract: The bark beetle, *Ips typographus* (Coleoptera: Scolytidae), is one of the most important pest insects in coniferous forests of Georgia as well as of Germany. In the last years, numerous investigations were focused on the pathogen complex of bark beetles and its possibility to impact on insect population dynamics. For our investigations, living adult *I. typographus* were collected from pheromone traps of two locations of Georgia (Borjomi gorge-Libani, district 16; Tsagveri, district 9; and Racha, Shovi) in summer of 2003-2004, and of three locations in Germany (forest districts of Hessen: Reinhardshagen with four areas, Darmstadt with four areas; forest district of Bavaria: National Park Bavarian Forest, Frauenau, with five areas) in summer 2005. The beetles were removed daily, determined, dissected and checked for infections with pathogens with the light microscope. The following antagonistic groups were investigated: Protozoa, fungi, mites and nematodes. In Georgian bark beetle populations, the entomopathogenic fungus *Beauveria bassiana*, the phoretic mites *Dendrolaelaps quadrisetus*, *Macrocheles* sp. (no. 48138), *Lasioseius penicilliger*, and *Trichouropoda polytricha*, *Histiogaster ornatus*, *Dendrolaelaps apophyseus*, *Ereynetes propescutulis*, and the nematodes *Contortylenchus* sp., *Parasitorhabditis* sp., and *Bursaphelenchus* sp. were found. In German populations of *I. typographus*, the microsporidium *Chytridiopsis typographi*, the fungi *Beauveria brongniartii*, *B. bassiana*, and *Fusarium* sp., and ten species of phoretic mites (*Dendrolaelaps quadrisetus*, *Proctolaelaps fiseri*, *Macrocheles* sp. (no. 48249), *Lasioseius penicilliger*, *Pleuronectocelaeno barbara*, *Histiostoma piceae*, *Uroobovella ipidis*, *Trichouropoda polytricha*, *Trichouropoda* sp. (no. 23936), *Tarsonemus minimax*) were identified. Furthermore, several undetermined nematodes were observed.

The challenge of controlling multispecies white grub associations

S. Keller, Y. Dhoj G.C., C. Schweizer..... 251

Abstract: White grubs (Scarabaeidae, Coleoptera) are important soil pest insects worldwide. In Central Europe only a limited number of species is present and damages are normally caused by

a single species. The presence of more than one damaging species is rare but seems to increase. In Nepal and probably elsewhere, a high species diversity exist and damages are usually done by multispecies associations. Most common pathogens of white grubs are fungi, especially *Metarhizium anisopliae*, *Beauveria bassiana* and *B. brongniartii*. Most white grub species are susceptible to *M. anisopliae*, but only a few ones like *Melolontha* spp. can be controlled with *B. brongniartii*. Therefore, the control of species complexes containing *Melolontha* sp. needs treatments with both fungi. Separate applications of *M. anisopliae* and *B. brongniartii* and a mixed application of these two fungi were done in Switzerland.

The results in respect to fungus development in the soils as well as to control efficacy were not consistent. There are indications that a fungus can inhibit the other one but there seems to be no generalised mutual inhibition of the two fungi. Consequently, good *Melolontha* control was also achieved in plots treated with both fungi. Probably soil characteristics and host densities influence the fungus development. The situation in Nepal is different. All white grub species tested so far are susceptible to *M. anisopliae*. The question that needs to be answered is: Can all damaging species of white grubs be controlled with the same isolate irrespective of their life cycles, the soil types and other environmental conditions?

Sublethal effects of *Nomuraea rileyi* on development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

C. Espinel, A. Cotes 257

Abstract: *Nomuraea rileyi* represents a promising biocontrol agent of fall armyworm *Spodoptera frugiperda*. Based upon a native strain Nm005, a biopesticide prototype, which produced mortality under laboratory conditions of *S. frugiperda* upper 90%, has been developed. Taking into account the importance of studying pathogen-insect interactions as a crucial factor for optimization of biopesticides, the objective of this study was to evaluate sublethal effects of *N. rileyi* Nm005 over biological parameters of *S. frugiperda* immature stages and adults. Nm005 was applied to eggs and second and fifth instar larvae in a lethal concentration 30 (LC₃₀), (2.4 x 10³ conidia ml⁻¹) to determine the effect through a generation. Larval mortality, longevity and weight of pupae were evaluated. Surviving adults were mated and the fecundity and fertility were determined. The same parameters were registered in the next generation and each developmental stage was compared with a control. Second instar larvae were the most susceptible to LC₃₀ of *N. rileyi*, with 36.3% mortality, compared to treatments of eggs or fifth instar larvae, with 17.9% and 13.2%, respectively. A sublethal effect was evident in fertility of surviving adults when second instars had been treated with the fungus (50.5% fertility) in comparison when eggs and fifth larvae instar had been treated (80.6% and 83.4%, respectively). Sublethal effects were evident in adults of the second generation obtained after treatment of eggs and second instar.

Nematodes

Isolation and characterization of new populations of entomopathogenic nematodes from Israel

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A new steinernematid epn from Sardinia island (Italy)

E. Tarasco, Z. Mráček, K. B. Nguyen, O. Triggiani 264

Abstract: A new *Steinernema* species was isolated from three different sandy soil samples along the Platamona Beach, in the north-west coast of Sardinia island (Italy). This new species is characterised by the following morphological characters: infective third-stage juvenile with a body length of 866 ± 66 (767-969) µm, distance from head to excretory pore of 63 ± 2.7 (59-68) µm, tail length of 81 ± 3.2 (76-89) µm, ratio E (%) 77 ± 3.4 (68-83); male tail with a mucron only in the second generation, spicule length of 66 ± 1.4 (64-67) µm and gubernaculum length of 44 ± 1.4 (43-46) µm in the first generation male; female of first generation with a slight vulval protruding and ratio D (%) of 50 ± 8.6 (29-65). The new species differs distinctly from the related species (*S. feltiae*, *S. kraussei*, *S. litorale*, *S. oregonense*) in some morphometric values and the analyses of the internal transcribed spacer (ITS) and D2D3 regions. The new

species is close to *S. feltiae*, *S. weiseri* and *S. litorale* based on the sequence of the ITS region. For the D2D3 region, it is close to *S. feltiae*, *S. oregonense* and *S. kraussei*. Sequences of D2D3 of other species are not available for the studies. Cross hybridisation tests with *S. feltiae*, *S. litorale*, *S. weiseri* and *S. oregonense* showed that these species were reproductively isolated.

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Abstract: *Steinernema affine* was recorded from an arable field, located near the village Staro selo, which had been cultured with cabbage the year prior to isolation of the strain. Future work can now test this nematode in outdoor field experiments against major cabbage pest insects. Cole crops are of major importance for Slovenian agriculture.
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M. Zec-Vojinovic 271
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- Entomopathogenic nematodes for control of codling moth: efficacy assessment under laboratory and field conditions**
S. Mouton, D. Juan, P. Coulomb 281
Abstract: Strategies to reduce codling moth populations in organic orchards may become inefficient in the close future as resistance has recently been observed against the granulosis virus, the major control strategy in organic farming. Entomopathogenic nematodes (EPN)

have shown interesting results as biological control agents and could be a way to reduce codling moth populations. The efficacy of two EPN species (*Steinernema feltiae* and *Steinernema carpocapsae*) was evaluated using different exposure methods, against various life stages of the codling moth under laboratory and field conditions. Nematodes can reach the fifth instar diapausing larvae in the ground within their cryptic habitats which currently provide them protection from extreme environmental conditions. The susceptibility of fifth-instar codling moth larvae was assessed under laboratory conditions using cocooned larvae within cardboard strips. The codling moth is significantly more susceptible to *S.carpocapsae* than to *S.feltiae*. The LC₉₀ values for *S.feltiae* and *S.carpocapsae* were 78.8 and 3.5 infective juveniles/cm², respectively. A field trial was also conducted in an apple orchard. EPN were applied on the soil, against cocooned larvae within cardboard strips in the ground. A test on neonate larvae was carried out to assess the potential efficacy of foliage spraying of nematodes, using two different exposure substrates. Even with a short time exposure, first-instar larvae may be more susceptible to nematodes than fifth instar diapausing larvae. Neonate larvae were exposed to EPN on wheat germ-starch artificial diet, and on young apples soaked in nematode solutions under laboratory conditions. The results will define the optimal conditions to use nematodes in codling moth control strategies: target pest stages, nematode species and concentration.

Effectiveness of entomopathogenic nematodes in the control of *Cydia pomonella* overwintering larvae in Northern Italy

A. Reggiani, G. Curto, S. Vergnani, S. Caruso, M. Boselli 287

Abstract: Research on the effectiveness of entomopathogenic nematodes (EPN) in the control of codling moth (*Cydia pomonella*) overwintering larvae have been performed in some pear orchards of Emilia-Romagna, Northern Italy. The evaluation of *Steinernema carpocapsae* and *Steinernema feltiae* activity in the larval control was checked after EPN foliar applications. The treatments were applied either in autumn (2004-2005) or in spring (2005-2006) before the codling moth pupation at optimal temperature (>12-14 °C) and after or during a rainfall or an irrigation. EPNs were applied at doses of 1.3 x 10⁹ IJ ha⁻¹, 1.7 x 10⁹ IJ ha⁻¹, 2.5 x 10⁹ IJ ha⁻¹ and 1.25 x 10⁹ IJ ha⁻¹. The larval mortality was assessed on sentinel larvae placed on band traps of corrugated cardboards tied to the trunks (autumnal and spring trials) and indirectly on the eggs laid by the females of the first codling moth generation (2006 spring trial).

Foliar application of EPNs to control *Xanthogaleruca luteola* Müller (Coleoptera, Chrysomelidae)

O. Triggiani, E. Tarasco 294

Abstract: *Xanthogaleruca luteola* Müller (Coleoptera, Chrysomelidae) is widely spread in Europe and North America and it is a dangerous pest of elm-trees. In Italy the elm-trees are often located in urban areas so the potential of 3 entomoparasitic nematodes (EPNs; 2 Italian strains of *Steinernema carpocapsae* and *S. feltiae* and 1 commercial strain of *Heterorhabditis bacteriophora*), was evaluated through field experiments for control of *X. luteola*. During the first 2 weeks of June 2006, *S. carpocapsae* (ItS-MR7), *S. feltiae* (ItS-CL2) and *H. bacteriophora* (Nematop®) in gel suspensions (with Idrosorb SR 200 and Xanthan gum) were sprayed on leaves and small branches heavily infested by *X. luteola* larvae in a periurban park of Bari city (Apulia Region, South Italy). The efficiency of these EPNs was then evaluated after 3 and 5 days. *S. carpocapsae* in Idrosorb + Xanthan gum controlled 67% of the larvae after 5 days, producing significantly greater larval mortality than *S. feltiae* and *H. bacteriophora*.

Cultivation conditions of biocomplexes applicable to control *Melolontha melolontha*

C. Sisak, Z. Kaskötő, T. Tóth, T. Lakatos 297

Abstract: Recently several nematode/bacterium complexes have been isolated in Hungary with potential to grubs of the European cockchafer (*Melolontha melolontha*). The cultivation of one of the most promising biocomplexes, *Heterorhabditis downesi* '267' + *Photorhabdus temperata* in liquid medium can be considered to be effective if the concentration of the dauer juveniles achieves 10,000/ml. A prerequisite for the elaboration of suitable conditions for cultivation is the development of optimal process conditions (aeration at low shear stress, medium composition, etc.) and to avoid the occurrence of phase variants of the bacterium symbiont.

Cultivation experiments with *P. temperata* were performed first in shake flasks, in LB and TSY medium at 20-22°C. The aeration rate required by the cells during the logarithmic growth period was about 0.6-0.7 vvm. Applying a 5 litre mechanically stirred fermenter equipped with draft-tube for bacterium growth studies, a special fedbatch technique has been elaborated. Cell numbers of the primary phase variant were sustained at high numbers over a period of 19 days. Medium optimization studies exchanged several protein components of LCM for lipid components and obtained considerably higher numbers of dauer juveniles and hermaphrodites. Rape seed oil was the most suitable lipid component. In the best medium, the initial nematode dauer juvenile number (1,000/ml) increased to 120,000/ml within 12 days. At present, scale-up studies of the biocomplex are in progress in column bioreactors of different type.

Recovery of *Steinernema carpocapsae* and *Steinernema feltiae* in liquid culture

A. Hirao, R.-U. Ehlers 301

Abstract: The development from infective Dauer juvenile (DJ) to third stage juvenile, so called “recovery”, occurs at almost 100% when DJs infect a living insect. In contrast, recovery is much lower, when DJs are inoculated in liquid culture media, in which the symbiotic bacteria were preincubated.

For the purpose of obtaining an increase in recovery in liquid culture, recovery inducing factor for *S. carpocapsae* and *S. feltiae* was investigated. The influence of penetration behavior on recovery was checked. DJs injected into *G. mellonella* or inoculated into sand containing the insect recovered at almost 100%. The penetration activity is excluded to influence recovery of *S. carpocapsae* and *S. feltiae*. The high recovery in insects might be due to a food signal present in the serum of *G. mellonella*. Inoculation of DJs in purified and antibiotics-treated serum was investigated. Whereas in vivo recovery of *S. carpocapsae* reached 100%, only 40% of *S. feltiae* recovered in sterilize serum. Whereas a food signal triggering recovery of *S. carpocapsae* is found in the serum, the composition of the *G. mellonella* serum seems suboptimal for recovery of *S. feltiae*. The influence of the density of the symbiotic bacteria, *X. nematophila* and *X. bovienii* (isolated from *S. carpocapsae* and *S. feltiae*, respectively) was investigated. The bacteria were proliferated in liquid medium for 36 and 48 hours. Then DJs at a density of 1,000 DJs/ml were inoculated at bacterial densities of 10⁸, 10⁹ and 10¹⁰ cells/ml. Recovery of *S. carpocapsae* was checked every two hours until 12 h post inoculation (hpi) and *S. feltiae* every two hours between 4 and 10 hpi. Comparing recovery at different bacterial densities, recovery of both nematodes increased with increasing bacterial density. However, the recovery of *S. feltiae* was delayed and lower compared to results obtained with *S. carpocapsae*. Culture supernatant of both bacterial symbionts contained food signal, however, the recovery was significantly higher when bacterial cells were present.

Differential gene expression of recovery in *Heterhorabditis bacteriophora*

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Expression of different desiccation tolerance related genes in various species of entomopathogenic nematodes

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Genetic improvement of beneficial traits mixed population of *Steinernema feltiae* for enhancement of persistence and efficacy

M. Chubinishvili, L. Salame, C. Chkhubianishvili, I. Glazer (Abstract only) 309

Soil insect pests and miscellaneous

When should we use biocontrol agents against leatherjackets (*Tipula paludosa* Meig.)

R.P. Blackshaw 313

Abstract: Recent work has demonstrated that biological control agents directed against the larvae of *Tipula paludosa* (leatherjackets) are more effective if applied against early instars in October rather than later instars in the late winter/early spring. In this paper simulation modelling is used to identify potential strategies for the deployment of biocontrol agents against leatherjackets in continuous grass, and in grass/arable rotations. It is concluded that the optimal prophylactic strategy in continuous grass lies between annual and biannual application

frequencies, and that application in the autumn preceding cultivation for a spring crop suppresses populations better than applications earlier in the rotation. A decision tool based on economic thresholds is presented for grass/arable rotations. The results are discussed and it is concluded that development of a robust monitoring system is necessary if recommendations other than prophylactic applications are to be made.

Biocontrol of *Capnodis tenebrionis* (L.) (Col., Buprestidae) with entomopathogenic fungi

P. Marannino, C. Santiago Álvarez, E. de Lillo, E. Tarasco, O. Triggiani, E.

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Abstract: A study was carried out at ETSIAM to investigate the potential of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok. for the control of the peach root-borer, *Capnodis tenebrionis* (L.), a major threat to stone-fruit orchards in several Mediterranean countries. The pathogenicity of 4 *B. bassiana* and 4 *M. anisopliae* isolates (from ETSIAM collection) against *C. tenebrionis* neonate larvae was assessed under endophytic conditions. Mortality rates varied from 23.5 to 100%, 10 days after inoculation by dipping in a suspension with 10^8 conidia/ml. The most virulent isolate, *M. anisopliae* EAMa 01/58-Su, was used as spore suspension (10^6 , 10^7 , 10^8 conidia/ml) for soil application on *Prunus myrobalana* Lois. potted seedlings 2 days before infesting them with *C. tenebrionis* neonates. The treatment caused a significant mortality (83.3 to 91.6%) on the larvae recovered from the roots 4 weeks later. No significant difference was detected among the doses. The susceptibility of *C. tenebrionis* adults to EAMa 01/58-Su was also assessed and a significant mortality (86.7%) was ascertained after immersion in a spore suspension (10^8 conidia/ml). No significant difference was pointed out between male and female mortalities. The effectiveness of the selected *M. anisopliae* isolate was proved also using non-woven fiber bands impregnated with conidia. Mortality of beetles that crossed the band varied from 85.7 to 100%. No significant correlation was found between time needed to cross the band and survival times. Our results demonstrate that EAMa 01/58- Su is suitable to target successfully both peach-borer stages.

Infectivity of *Steinernema feltiae* and *Heterorhabditis bacteriophora* towards first and second stage larvae of the forest cockchafer, *Melolontha hippocastani*

K. Jung, A. Peters, J. Pelz..... 327

Abstract: At high population densities, the forest cockchafer, *Melolontha hippocastani* is a serious pest insect causing death to trees and seedlings. In the southern Rhine valley of Germany, a new gradation of *M. hippocastani* has built up since the 1980s. Despite considerable results on the control of grubs with entomopathogenic nematodes (EPN), so far no nematode was identified, which efficiently infect *Melolontha* spp. We herein report on laboratory assays with *H. bacteriophora* and *S. feltiae*, both alone, in combination with one another and with *B. brongniartii* against the 1st and 2nd instar larvae of *M. hippocastani*. First instar larvae did not seem to be more susceptible than second instar larvae. None of the nematode, alone or in combination caused high mortality, with *H. bacteriophora* being a little better than *S. feltiae* (28 compared to 4 % at 500.000 IJ's/m²). In conclusion, the nematodes currently available on the German market cannot reach control reported for *B. brongniartii* (BIPESCO1; 60 % at 1×10^{10} conidia/m²).

Susceptibility of soil-dwelling developmental stages of rose-infesting sawflies to entomopathogenic nematodes

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Field results on the use of *Heterorhabditis bacteriophora* against the invasive maize pest *Diabrotica virgifera virgifera*

R.-U. Ehlers, U. Kuhlmann, S. Toepfer 332

Abstract: The western corn rootworm (CRW) (*Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) is an invasive alien pest of maize (*Zea mays* L) in Europe. The three larval instars feed on maize roots and cause the major damage. Chemical control measures usually reduce the root damage by one unit on the Iowa root rating scale. Among several different nematode species tested in the laboratory, *Heterorhabditis bacteriophora* (Nematoda: Rhabditida) was selected as the most efficient species against larvae of the CRW and was

further tested as a biological control product for inundative releases against *D. v. virgifera* larvae. The control efficacy of *H. bacteriophora* was studied in eight maize fields in southern Hungary between 2004 and 2006. Nematodes were either applied in May during sowing of the maize with the precision seed drill machine or in June when plants were >30 cm high and larvae had hatched with conventional spraying equipment. In May nematodes were applied in liquid or powder into the soil in the row where the seed was deposited. After application the machine covered nematodes and seeds with soil. Application in June was in liquid by solid stream application along both sides of the plant or over the complete field with flat fan nozzles. Damage and number of emerging adults per plant was assessed in July. In untreated control plots, 23% of plants surpassed the economic root damage (Iowa root rating index >3). In treated plots the percentage of plants with economic damage was between 5.6 and 8.6%. The nematode treatment reduced the mean total root damage by 70 %. The mean number of adults per plant was 1.7 in the control plots and 0.3 in plots applied with nematodes in liquid in May (81% reduction). The June application was often less effective than the applications in May and application into the soil (May) or solid stream application (June) were more successful than coverage of the whole field with flat fan nozzles.

Pest status of *Tecia solanivora* (Povolny 1973) (Lepidoptera: Gelechiidae), Guatemalan Potato Moth, in the Canary Islands

A. Carnero, A. Padilla, S. Perera, E. Hernández, E. Trujillo 336

Abstract: First reports of potato damage by *Tecia solanivora* in the Canary Islands date back to 1991, in Tenerife. Although continental Europe is currently free of *T. solanivora*, it is an important quarantine pest elsewhere, and its spread to other islands posed a serious threat to the local staple potato crop. Initial presence was established by placing pheromone traps in several field localities and in wholesale seed-potato warehouses throughout Tenerife. December 2006 data showed that the entire northern side of the island is affected, while infestations in the south are restricted to the Güimar Valley area (Güimar, Fasnia and Arico counties). Its presence on the islands of Gomera, Grand Canary and La Palma is also reported.

Development of microsatellite markers for the assessment of population structure of the European cockchafer *Melolontha melolontha*

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Protozoa, fungi and nematodes in *Gastrophysa viridula* (Coleoptera, Chrysomelidae) from Austria and Poland

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Occurrence of protozoan pathogens in *Hylobius abietis* L. (Col., Curculionidae) from Austria, Poland and Sweden

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Bioassays with entomopathogenic organisms on adult *Cosmopolites sordidus* (Germar, 1824) (Coleoptera: Curculionidae)

A. Padilla Cubas, A. Carnero Hernández, L. V. López-Llorca, F. García del Pino (Abstract only) 343

Biological control of *Thrips tabaci* in the field – possibilities and practical limits

K. Jung 344

Abstract: Field trials against *Thrips tabaci*, an economically important pest insect, were conducted in 2003 at four different sites in Germany. The effect of the combined use of entomopathogenic nematodes and fungi on thrips should be investigated, in comparison to the effect of either pathogen alone. Commercially available products, Mycotal® (Koppert NL), PreFeRal® (Biobest BE), Naturalis L® (Intrachem IT), Nemaplus® and NemaGreen® (both E-Nema DE), were used. The trials were performed in onion, leek and chives, according to the EPPO guideline PP 1/85(3). Perfekthion® was used as chemical standard in onion. In leek, Spruzit® and Neudosan® were applied alternately. No chemical standard was applied in chives. The biocontrol products were applied either alone or in a mixture using common spray equipment. The nematodes were applied at a rate of 1x10⁶ infective juveniles/m². 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.

Insect pressure was low at all four sites, throughout the summer. *T. tabaci* was recorded only in medium numbers (mean of 30 specimen/plant max.). In two trials, no differences were detected between the treatments and the control. In the third trial (onion), 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 (3 times in total). Also in the fourth trial (leek), the number of thrips/plant was lowest for the treatments 'fungi+nematode' (4 compared to 7 in the control), one week after the final application (6 times in total). In this trial, yield was measured additionally, and the weight/plant registered at harvest was 20 % higher for the treatments 'Nemaplus' and 'Mycotal+Nemaplus' than in the control (425 and 412 g/plant compared to 345 g respectively).

The results presented here, show that the use of biocontrol nematodes and fungi need not be restricted to protected environments, field application against insects on the upper parts of plants is possible. Moreover, their combination has the potential to give a better reduction of the pest population than each agent alone. In consideration of the extreme weather conditions in Germany in 2003, and in comparison to the effect of the chemical standards, the results are regarded as promising. However, each crop situation needs its own proof of feasibility, since factors like the microclimate, created by the plant's individual characteristics and the canopy it forms, may influence the activity of the pathogens and by this their efficacy.