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**Working group "Insect Pathogens and
Insect Parasitic Nematodes"**

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les Nématodes Parasites d'Insectes"**

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Molecular approaches for diagnostics and phylogenetics of entomopathogenic nematodes: applications and implications for pest management

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Abstract: Several molecular approaches are currently used in support of biological control as fast and cost effective ways of diagnostics and in analyzing data on relationships of both the target pests and the agents involved in their control. With respect to entomopathogenic nematodes (EPN), a variety of molecular methods have been used to diagnose, delimit species and infer their evolutionary histories. Of all, DNA sequence analysis has demonstrated to yield more information about variation within and among nematode species than other methods previously used. Furthermore, analysis of sequence data has shown to be a more suitable tactic in assessing phylogenetic relationships at different taxonomic levels, as well as for delimitation of species, and in separating cryptic species. In spite of these accomplishments, there still are many milestones and challenges that need to be achieved and unravelled in the study of EPN molecular biology. For example, until now, very little progress has been made in understanding the genetics of EPN at the infraspecific level. Yet, it remains to be defined what we understand as "populations" and delimit the geographic boundaries of such populations. This level of discrimination is yet much needed not only to meet possible requirements of registration for isolates but also to provide verification tools for proprietary rights to patented nematodes. For this, it is expected that new advances in molecular biology and comparative genomics will significantly expand our gamut of molecular markers and analytical tools. A summary of the current molecular approaches for the study of EPN is herein presented and discussed.

Control of Codling Moth, *Cydia pomonella* (Lepidoptera: Tortricidae) with nematodes (*Steinernema* spp. and *Heterorhabditis* spp.)

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Abstract: As entomopathogenic nematodes are known parasites of *Cydia pomonella* under natural conditions, several trials were conducted to determine the most efficient nematode species and to evaluate its efficacy in the field. In laboratory experiments the LD₅₀s caused by the four nematode species *Steinernema carpocapsae*, *Steinernema feltiae*, *Heterorhabditis bacteriophora* and *Heterorhabditis megidis* were not significantly different. However *Steinernema carpocapsae* appeared to be more effective than *Heterorhabditis megidis* in a semi field essay. Unfortunately, due to a low retrieval rate of the initially released insects, this result was based on only very few individuals. Further, in autumn 2004, trunks of apple trees in an orchard in Southern Germany were treated with *Steinernema carpocapsae*. One month after the treatment diapausing larvae of *Cydia pomonella* in the trunk bark were isolated and the mortality caused by nematodes was assessed. In the untreated control 13% were infected with nematodes, whereas a proportion of 42% larvae infected with nematodes was noticed in the treated plot had.

Effectiveness of entomopathogenic nematodes in the control of sawfly (*Hoplocampa brevis*) in pear orchards

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Abstract: *Hoplocampa brevis* (Hymenoptera, Tenthredinidae) has caused recently severe damages to pear fruits in Italy, particularly in organic orchards, leading to heavy economic losses. A product based on *Steinernema feltiae*, suitable for leaf treatments, was applied for the control of sawfly larvae once or twice at different doses (250,000 IJ/m² and 500,000 IJ/m²); nematode based insecticide was compared with one rotenone treatment and with a product based on rock powder and plant oils. Furthermore, in the same trial, in an area of 1 m² repeated four times, under three trees which were not treated on the leaves, two products based on *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* were applied to the soil just before the drop of sawfly mature larvae from the fruits. The results showed interesting possibility of entomopathogenic nematodes (EPNs) applications in the sawfly management of organic farms, because in the plots treated with foliar applications of *S. feltiae*, the percentage of infested fruits was the same as the one obtained with rotenone (10-25%) and moreover, in a good percentage of young pears (about 40%), EPNs were found eight days after the last treatment. The soil application of EPNs registered a good effectiveness in the control of sawfly larvae, reducing significantly the adult population in the next spring.

Nematode cryopreservation using a mechanical freezer at -140°C: a preliminary report

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Abstract: Temperatures below -130°C are known to assure long-term, and possibly indefinite preservation of various biological specimens. Recently, the *Bursaphelenchus* spp. I.S.Z.A. collection has been enlarged much by the acquisition of new nematode populations, referred to *B. eremus* (Rühm) Goodey. This species was isolated in Italy both from dying *Quercus* spp. and also from the bark beetle *Scolytus intricatus* Ratzeburg as dauer larvae. So, a cryopreservation technique using a mechanical freezer at -140°C has been developed for a long-storage of all the *Bursaphelenchus* spp available.

Effect of *Melolontha melolontha* grubs on persistence of entomopathogenic nematodes in soil

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Abstract: Effects of *Melolontha melolontha* grubs on persistence of three entomopathogenic nematodes, namely *Steinernema glaseri* (NC), *S. arenarium* (Ryazan) and *Heterorhabditis megidis* (01PL), were tested in two laboratory bioassays. The first test was carried out in PCV pots filled with a moist peat. The nematode infective juveniles (IJ) were applied at the rate of 10^6 m⁻² per grub. The grubs were then withdrawn from the containers 8, 14 and 21 days after nematode application, examined for mortality and dissected to evaluate the number of nematodes present inside. All withdrawn grubs were replaced with 5 caterpillars of *Galleria mellonella* per container. After additional 72 h of incubation, the caterpillars were dissected and examined for the presence of nematodes. In general, the survival of nematodes was better in containers with no grubs. The greatest number of nematodes was observed in pots with *S. arenarium* (Ryazan) (mean 137.8 per pot), while the lowest one was in pots with *H. megidis* (01PL) (mean 37.4/pot). In the second bioassay, conducted in Petri dishes, moist sand was inoculated with 100 infective juveniles of *S. glaseri* (NC). To assess nematode persistence in presence of a grub, IJs were extracted from the sand using a decant and sieve technique. The mean number of living nematodes was significantly highest in absence of grub (61.6/dish) than in combination with the grub (53.5/dish). Persistence of tested nematodes varied according to time of their exposure, nematode species and presence or absence of the grub.

Effects of UV-B radiation on *Steinernema apuliae* (Rhabditida: Steinernematidae)

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Abstract: The effects of UV exposure on surviving entomopathogenic nematodes have been remarkably documented; however, the effects of UV exposure on *Steinernema apuliae* are unknown. In the bioassay conducted, a UV lamp was used that emitted medium wavelength radiation because UV-C light is known to rapidly kill entomopathogenic nematode species. The experiments were carried out on six strains of infective juveniles of *S. apuliae* in water. Each strain was exposed for 0 (control) and 5 and 10 minutes at different distances from the lamp with three replications. With the objective to determine the survival rates for each strain, the nematode mortality rates were analyzed after 1, 3, 5, 7, and 14 days. The survival curves were also compared to evaluate the significant difference among the strains. The ability of the irradiated infective juveniles to determine lethal infection was later tested against last-instar *Galleria mellonella* larvae.

Efficacy and environmental impact of entomopathogenic nematodes used against nut insect pests in some chestnut woods on Etna (Italy)

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Abstract: Investigations were carried out to test the possibility of controlling the main chestnut fruit insect pests (tortricid caterpillars and curculionid larvae) on Mount Etna (Italy) by means of entomopathogenic nematodes (EPN) belonging to the genera *Steinernema* and *Heterorhabditis*. A survey of autochthonous EPN species, which should more suitable to be used locally, was conducted in many chestnut groves of Etna and various populations of nematodes, mainly belonging to *Steinernema*, were found. Laboratory tests were made to evaluate the ability of several EPN species and strains to parasitize larvae: most EPN species were shown to be effective against most insect larvae extracted from the nuts. In particular, *H. bacteriophora* showed a great efficacy both against the tortricids and curculionids. Soil insemination with different EPN species was made to test their persistence ability in the soil. The most persistent species was *H. bacteriophora*, which was found in the soil for many months after the soil insemination, that is for a time sufficient to let these nematodes find and kill the insect larvae in the soil. The environmental impact of the EPN insemination on the soil fauna was also thoroughly studied and no significant differences were found in the soil fauna composition before and after the treatment.

Incidence of natural infection of the white grub *Polyphylla olivieri* (Coleoptera: Scarabaeidae) with entomopathogenic nematodes in Iran

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Abstract: From June 2004 to August 2005, larvae of the white grub *Polyphylla olivieri* and soil samples were collected in fruits orchards in seven counties of the Tehran province, Iran. Whites grubs were reared in insectarium, and monitored for possible infection by EPN and subsequent isolation of the nematodes. Isolation of EPNs from soil samples were conducted using the *Galleria* bait method. Three species of EPNs were identified: *Heterorhabditis bacteriophora* (IRAN1), *Steinernema glaseri* (IRAN2) and *Steinernema* sp. (IRAN3). In bioassays against the White grub, the LD₅₀ of IRAN1 was 35 IJs/larva, followed by 65 IJs/larva for IRAN2. The LD₅₀ for *Steinernema* sp was >10000 IJs/larva and caused only 16% mortality after 25 days. Tolerance of the three Iranian EPNs (IRAN1 of *H. bacteriophora*, IRAN2 of *S. glaseri* and IRAN3 of *Steinernema* sp.) were compared. Heat tolerance study showed that the *H. bacteriophora* strain was the most tolerant nematode at 32°C, but no nematodes could survive at 36°C after a 4 – 5-h exposure. Furthermore, life cycle and natality/mortality data of the three Iranian isolates were studied in the wax moth larvae, *G. mellonella*, at a range of temperatures from 5 to 30°C.

Biological characterization of *Steinernema apuliae*: first contribution

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Abstract: Eight strains belonging to *Steinernema apuliae* were collected from soils of different biotopes in Southern Italy during the last 10 years. Bioassays were performed in laboratory with the aim to assess some biological aspects of this nematode: the performance of the nematode against different species of insects, i.e. *Bactrocera oleae* (Diptera: Trypetidae), *Sarcophaga carnaria* (Diptera: Sarcophagidae), *Corythucha ciliata* and *Monosteira uncostata* (Rhynchota: Tingidae), and *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) in comparison with the infectious activity of *S. carpocapsae* (ItS-MR7 Italian strains); the infectious behaviour depending on different temperatures using *Galleria mellonella* larvae as test insect; the desiccation tolerance upon exposure to 2 relative humidities (84% and 76% RH); the optimum storage conditions with different concentrations at different temperature levels (8°-12°-16°-20°C).

Molecular characterization of Italian EPN strains by RFLP analysis of the ITS region of the ribosomal DNA repeat unit

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Abstract: The ITS region of 51 Italian strains of entomopathogenic nematodes (EPNs) belonging to 4 species isolated in the last 10 years in Southern Italy was amplified by PCR and the resulting products were digested with 9 different enzymes; the fragments generated were then separated by agarose electrophoresis. The strains were previously identified upon morphological examination using morphometric data. For many of the strains RFLP analysis confirmed morphological identification (*Steinernema feltiae*, *S. affinae*, *Heterorhabditis bacteriophora*), whereas for some RFLP analysis evinced different data from the morphometric examinations. In particular, the RFLP profiles of 3 strains from Sardinia showed differences compared with the profiles of the known species and were quite similar to a strain called MY2 (*S. litorale*), which until now has only been found in Japan.

Efficacy of *Steinernema carpocapsae* against the cockroach *Periplaneta australasiae*

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Abstract: Cage tests showed that the nematode *Steinernema carpocapsae* is able to infect and to kill the cockroach *Periplaneta australasiae*. Mortality of 85 % was reached using different traps. To lure the cockroaches into the traps different baits and foods were offered. Coffee and banana were as good as commercial products. Besides banana was the preferred food. When nematodes were applied to cockroaches for two minutes 80 % of exposed insects died. The mortality increased even to 95 % after 30 minutes. Moreover the number of nematodes on the cockroach's body was counted. Cockroaches in contact with nematodes for ten minutes had average 431 *S. carpocapsae* on their bodies. If the cockroaches were isolated after the confinement for 24 hours the number of nematodes amount to 147. We suppose that the nematodes enter the host through the spiracles: 83% of the cockroaches died if the nematodes were placed at the side of thorax and abdomen, compared with lower mortality when applying the nematodes on other places (65% dorsal, anus 38%, ventral 33%, mouth 25%).

Strategies to control woodlice with entomopathogenic nematodes

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Abstract: The efficacy of entomopathogenic nematodes in different formulations against woodlice was investigated. Individual *Porcellio scaber* and *Armadillidium* spp. woodlice treated with nematodes were more susceptible to *Steinernema carpocapsae* than to *S. feltiae*. Experiments with different nematode doses, nematode carriers and bait substances revealed that after 14 days incubation time, 86 to 100% mortality was reached when *S. carpocapsae* was formulated into quartz sand with compost as bait. Tests with cucumber plants indicated that older plants are protected from woodlice attack by the nematode bait formulation.

Entomopathogenic nematodes and forest insects in Italy

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Abstract: Entomopathogenic nematodes (EPNs) in Italy are used above all in greenhouses and nurseries. Usually not indigenous but commercial EPNs are used from northern European producers. Application of EPNs in forest biotopes is uncommon and only experimental trials have been conducted on few forest pests. It is important to consider that during the past 10 years, the occurrence of EPNs (Rhabditidae: Steinernematidae and Heterorhabditidae) was investigated in pine and oak woods in Southern Italy. Six percent of pine woods and 15.5% of oak woods were positive for the presence of nematodes. *Steinernema feltiae* was the most common (42%) species in both pine and oak woods. *S. feltiae*, *S. apuliae* and *Heterorhabditis bacteriophora* were collected in the pine woods and *S. feltiae* and *S. affine* in the oak woods. Many of the following indigenous EPNs isolated from forest soils were used in laboratory and field trials to control some dangerous insect pests of forest and urban trees in the Apulia region: *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae), *Corythucha ciliata* (Rhynchota: Tingidae), *Galerucella luteola* (Coleoptera: Chrysomelidae) and *Tomicus piniperda* (Coleoptera: Scolytidae). Indigenous EPNs were also used against *Balaninus* (= *Curculio*) *elephas* (Coleoptera: Curculionidae) in Sicily and the Lazio region, whereas indigenous and commercial strains of EPNs were used to control *Cephalcia arvensis* (Hymenoptera: Pamphilidae), *Zeuzera pyrina* (Lepidoptera: Cossidae), *Cossus cossus* (Lepidoptera: Cossidae), *Parenthrenea tabaniformis* (Lepidoptera: Sesiidae) and *Cryptorhynchus lapathi* (Coleoptera: Curculionidae) in the northern regions of Italy.

Chestnut pest control with entomopathogenic nematodes – prospects and constraints

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Abstract: Laboratory and field experiments were carried out to evaluate the biological control potential of entomopathogenic nematodes against the chestnut weevil *Curculio elephas*. Ten nematode strains were screened for virulence against *C. elephas* in the laboratory and four commercial nematode products were applied under semi-natural conditions in the field. In the laboratory, strains of *Steinernema bicornutum*, *S. feltiae* and *Heterorhabditis megidis* caused highest pest mortality. Soil applications of commercial nematode products into PVC tubes (depth: 40 cm) interred in a chestnut orchard showed no significant control effects. Reasons for lacking effects and possibilities to improve biological control of this pest are discussed.

Control of the hazelnut borer, *Curculio nucum*, with entomopathogenic nematodes

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Abstract: One of the most important insect pests for hazelnuts is the weevil, *Curculio nucum*. In laboratory trials using the sandy loam from the hazelnut orchard, three nematode species, *Steinernema carpocapsae*, *S. feltiae* and *H. bacteriophora* were applied to larvae collected in August 2002 in Cancon, France. The first assessment revealed 76%, 33% and 23% mortality 7 days after treatment with 100 infective juveniles/larvae of the three species, respectively. In a second experiment the *S. feltiae* that had propagated in the infected weevils were used and caused 88% mortality at a rate of 100 infective juveniles/larvae. A further trial was made in June 2004 with larvae from the same location using *H. indica* at rates of 5, 10 and 50 infective juveniles per cm². Larval mortality was >60% for all the doses tested. In field trials with artificially infected soil in buried buckets in hazelnut orchards, the mortality was 41, 65 and 75% in after applying *S. feltiae*, *H. indica* and *H. bacteriophora*, respectively, at a rate of 2.2 million/m², whereas 14% died in the untreated control. When repeating the experiment in 2004 with a dose of 0.5 million/m² larval mortality was not significantly different from the untreated control. Most larvae were found at a depth of 20 to 30 cm in the soil columns but some larvae were found down to 40 cm. Only 5% of the larvae were at 0 to 10 cm depth. All the nematodes applied did establish on the trial site as shown by baiting the soil with wax-moth larvae one year after treatment. However, most bait insect were infected in plots treated with *H. bacteriophora* which indicates the superior persistence of this species compared to *S. feltiae* and *H. indica*. The emergence of beetles two years after nematode treatment was significantly lower in the plots treated with *H. bacteriophora* or *S. feltiae*, while no difference to the untreated control was found in plots treated with *H. indica*. It is concluded that *H. bacteriophora* is the most promising candidate for controlling the hazelnut weevil pest to its good persistence and efficacy.

The effect of linearly polarized light on pathogenicity and reproduction in entomopathogenic nematodes

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Abstract: The aim of the study was to find out the effect of linearly polarized visible radiation on pathogenicity and reproduction in entomopathogenic nematodes. Exposure to light positively affected the pathogenicity of invasive larvae for test insect, *Tenebrio molitor*. The irradiated nematodes killed insects in greater numbers and more rapidly in comparison with the not illuminated ones (control). This positive effect of linearly polarized light on the pathogenicity of *S. carpocapsae* was not followed by better reproduction of the microorganism from dead insect bodies.

Laboratory trials of microbiological control of *Agelastica alni* L. with *Beauveria bassiana* (Bals.) Vuill.

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Abstract: The authors tested the entomopathogenicity of *Beauveria bassiana* isolates, obtained from forest soils, against the chrysomelid *Agelastica alni*, a pest of alder trees. In 2004, conidial suspensions of three isolates of the hyphomycete (BbaT01, BbaT06 and BbaT08) were adjusted in the laboratory to a concentration of nearly 3×10^6 cfu/mL and sprayed on the leaves of apical shoots of *Alnus cordata* (Italian alder). Controls were sprayed only with water. Treated and control shoots were placed on damp filter paper inside Petri dishes of 15 cm Ø with five *A. alni* various instar larvae. Each experiment was repeated four times. Dishes were kept under laboratory conditions and controlled daily. All *B. bassiana* isolates were entomopathogenic against *A. alni*, but showed differences in the survival time of the chrysomelid.

Entomopathogenic fungi isolated from various substrates in Italian pine woods

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Abstract: From 2001 to 2004, investigations were carried out in several maritime (*Pinus pinaster* Aiton) and nigra pine (*Pinus nigra* Aiton) stands of Tuscany (Italy) to isolate entomopathogenic fungi from various substrates (soil, bark and dead infected insects). Fungi were isolated in laboratory using different methods. The results showed a widespread presence of entomopathogenic fungi; in particular, *Beauveria bassiana* was obtained from all the substrates while *Paecilomyces* spp. were detected in both bark and insect samples. The entomogenous fungi *Lecanicillium evansii* and *Nectria inventa* were also recorded.

Does *Beauveria* spp. produce toxins after application when the product is present at the crop?

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Abstract: The question of possible production of toxins by *Beauveria* after applications seems to be utmost important especially for regulation authorities in Europe. Members of the RAFBCA consortium were confronted with these issues because little is published: (i) about the concentration range of metabolites produced by fungal BCAs; ii) about whether relevant metabolites enter the food chain, therefore posing a risk to human and animal health as well as the environment; (iii) about relevant studies about workers tested concerning exposure risk to toxins with focus on exposure to fungal products and to toxicologically relevant compounds in the products. As a matter of fact, there is sufficient information from literature which demonstrates that *Beauveria* does not produce relevant metabolites (toxins) during or after application. No risks to workers and bystanders are expected. There is no indication of environmental risk, nor do relevant metabolites enter the food chain. *Beauveria* spp. is therefore a safe biological control agent which should be registered in Europe without any restrictions.

Intraguild interactions involving the entomopathogenic fungus *Pandora neoaphidis*

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Abstract: Cage arenas were used to study the interactions between three natural enemies of the pea aphid, *Acyrtosiphon pisum*; the entomopathogenic fungus *Pandora neoaphidis*, the coccinellid predator *Coccinella septempunctata* and the hymenopteran parasitoid, *Aphidius ervi*. *A. ervi* and *C. septempunctata* significantly reduced populations of *A. pisum* when introduced as individual species whereas *P. neoaphidis* had no effect on aphid population size. Foraging by both *C. septempunctata* and *A. ervi* increased the abundance of *P. neoaphidis* which may be sufficient to initiate an epizootic. The benefits of increased transmission by *C. septempunctata* therefore outweighed the fitness costs to the fungus of intraguild predation. Although *P. neoaphidis* was associated with a decrease in the reproductive success of *A. ervi* in the cage arenas (where abiotic conditions were optimal for the fungus), preliminary experiments done under sub-optimal conditions indicated that *A. ervi* did not incur a fitness cost from foraging in patches containing *P. neoaphidis*.

Molecular tools to study natural occurrence, ecology and phylogeny of Entomophthorales

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Abstract: Molecular tools based on Polymerase Chain Reaction (PCR) technology provide opportunities to study the ecology and natural occurrence of entomophthoralean fungi in greater detail. Here, we give a short review on studies in which PCR technology has been implemented to investigate host range, geographic origin, life history traits and taxonomy of Entomophthorales. Preliminary results from some of author's current projects are also present.

Development of a new cultivation independent tool for monitoring the *Beauveria brongniartii* biocontrol agent in the field

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Abstract: Monitoring of applied fungal biocontrol agents traditionally depends on re-isolation and cultivation of the organism prior to identification. We have developed a cultivation independent PCR strategy for *Beauveria brongniartii*, the fungus used in biological control of *Melolontha melolontha*. For this the microsatellite tool used in the cultivation dependent approach has been adapted for direct application on bulk soil DNA samples.

Five soil samples obtained from a plot treated with the *B. brongniartii* BCA and five soil samples obtained from an untreated control plot have been investigated with the traditional cultivation dependent as well as with the new cultivation independent strategy. Both approaches confirmed the successful establishment of the applied BCA strain in the treated plot. The cultivation independent approach was shown to be reliable and it correlated with results obtained with the cultivation dependent approach. Yet, due to the smaller soil sample size used in the cultivation independent strategy the sensitivity is reduced as compared to the cultivation dependent strategy. The cultivation independent strategy provides a very efficient new approach, which allows to circumvent the time consuming cultivation step of the traditional monitoring strategy. The same approach can be used to analyse bacterial and fungal community structures, which eventually will allow to study potential effects of BCA application on other soil microbial populations in the same sample.

Fungal BCAs in the European Union: *Beauveria brongniartii* (Sacc.) Petch as the model organism to address key questions

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Abstract: Significant progress has been made in the development of fungal biocontrol agents (BCAs) for the control of insect pests. Nevertheless, no mycoinsecticide has yet been approved under European Union registration procedures. Critical issues in the registration of fungal BCAs are (i) the efficacy of the product, (ii) potential effects on non-target organisms and biodiversity, and (iii) the toxicity as well as risks posed by compounds synthesized by the microorganisms. *B. brongniartii* was used as a model organism to assess these issues and whether the ERBIC Risk Index would be adequate for the evaluation of fungal BCAs. It was demonstrated by accomplishing slight adaptations, the Index can be effectively used for the assessment fungal BCAs. The application of the ERBIC Risk Index to *B. brongniartii* resulted in a very low Risk Index of 15 on a scale of 125.

Preliminary survey for insect pathogenic fungi in Greenland

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Abstract: Data from a preliminary survey on natural occurrence of entomopathogenic fungi in Greenland are presented. The presence of Entomophthorales on adult flies is documented, as well as the presence of Ascomycetes (asexual stages) in soil samples.

Laboratory studies to assess the effects of the fungus *Lecanicillium lecanii* on the aphid *Schizaphis graminum*

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Abstract: The pathogenic effects of *Lecanicillium lecanii* ITEM 3757 towards winged and wingless adult morphs of the species *Schizaphis graminum* were investigated under laboratory conditions. Fungus was applied to aphids in the form of ground dried cultures grown in solid state fermentation on rice kernels. This formulation affected aphid survival and reproduction, and it interacted differently with winged and wingless aphid morphs. Scanning Electron Microscope observations pointed out a higher amount of adhering formulation and faster rates of fungal germination and sporulation on the winged cuticle. Our results suggest that the strain might be a good candidate for a programme of biocontrol against *S. graminum* and other aphids species.

Visualizing the infection process of the entomopathogenic fungi *Beauveria bassiana* and *Pandora neoaphidis* in aphids

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Abstract: *P. neoaphidis* and some *B. bassiana* isolates are pathogens of aphids. These fungi possess a great potential for microbial control of aphids, and knowledge about the infection processes can assist in the development towards biological control. The objective of this recently initiated study is to visualize and elucidate the infection process, especially penetration and colonization, of aphids by these fungi, using Confocal Laser Scanning Microscopy (CLSM). The aphid exoskeleton has autofluorescent properties and can easily be excited by ultraviolet or visible light in a CLSM. Experiments were done attempting to stain the fungal isolates with cfda (carboxy fluorescein di-acetate), Nile Red and Calcofluor white. In addition, *gfp*-tagging of *B. bassiana* using *Agrobacterium tumefaciens*-mediated transformation was conducted. *gfp* was inserted into two *B. bassiana* strains. Because the selective media were not sufficiently inhibiting the fungal growth, the selection for transformants was difficult and only a few transformants of each strain was found. We will continue our work with CLSM, staining and *gfp*-tagging to hopefully obtain 3D registration of the infection processes by entomopathogenic fungi in aphids *in situ*.

Naturally occurring *Beauveria bassiana* in *Hypothenemus hampei* populations in unsprayed coffee fields

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Abstract: Three unsprayed coffee fields in Nicaragua were studied for the natural occurrence of the insect pathogenic fungi *Beauveria bassiana* in *Hypothenemus hampei* populations throughout the rainy season in 2004. Infection levels were varying considerably throughout the season and between locations. The highest *B. bassiana* infection level observed at one location was 60%. This was observed in November at the end of the rainy season. Three moist chamber methods were used in the survey: a water agar based method, a filter paper based method and a plastic bag based method. The plastic bag method resulted in the highest and most frequent observation of *B. bassiana*. It is probably also the most reliable method when it comes to cross contamination.

Activity and molecular characterization of *Metarhizium anisopliae* isolates, of potential use for Moroccan locust control

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Abstract: *Dociostaurus maroccanus* is an important pest that can reach in Italy an alarming population level, causing extensive and severe damage to the crops. International programmes which were set up for biological control of locusts and grasshoppers, led to the isolation in Niger identification of a fungus as *Metarhizium anisopliae* var. *acridum*. This fungus became the most promising agent for biological control in Africa, and was later on tested in Europe, together with other species of fungi, such as *Beauveria bassiana*. During these trials a different isolate of *M. anisopliae* was found on locusts sampled in Gargano National Park, in Italy. The aims of this research were therefore: a) to test in the laboratory the effectiveness of two *M. anisopliae* varieties (i.e. *acridum* and *anisopliae*) against *D. maroccanus*; b) to characterize a representative strain of these two varieties by using ITS sequence analysis. The results were as follows: a) a higher mortality of locusts due to *Metarhizium anisopliae* var. *anisopliae* in respect of the var. *acridum*; b) the analysis of the ITS region of the two *M. anisopliae* varieties demonstrated its overall conserved character in this fungus. Nevertheless, some variability was detected within a portion (about 600 bp) of the rDNA gene complex, thus allowing the identification of the variety *M. anisopliae* var. *acridum* and *M. anisopliae* var. *anisopliae*.

Entomopathogenic fungi in riparian soils of the Ofanto river valley (Apulia region, Italy)

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Abstract: The natural occurrence of entomopathogenic fungi was investigated in different riparian biotopes of the Ofanto river valley situated in the center of the Apulia region (Southern Italy). During the period between September 2003 and June 2004, seventy-three different sites were sampled using the larvae of *Galleria mellonella* and *Tenebrio molitor* as bait insects. Fungi were isolated from 69 soil samples (94.5%) with 238 isolated strains belonging to 7 taxa.

Insect pathogenic fungi found in rosy apple aphid (*Dysaphis plantaginea*) and green apple aphid (*Aphis pomi*) in Norway

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Abstract: The natural occurrence of insect pathogenic fungi in *Aphis pomi* and *Dysaphis plantaginea* populations in apple orchards in south east Norway were studied throughout the summer 2002 and 2003. Four entomophthoralean species were observed to infect both apple aphid species: *Entomophthora planchoniana*, *Neozygites fresenii*, *Pandora neoaphidis* and *Conidiobolus obscurus*.

The effect of medium and selected metal ions on growth of the entomopathogenic fungus *Beauveria bassiana*

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Abstract: The effects of several culture media and selected metal ions (Cd, Pb and Zn) on growth of a few strains of *B. bassiana* was investigated on solid media in Petri dishes. Linear growth was found to be dependent on the medium as well as on strains. The highest growth occurred when strains were cultured on media without any addition of metal ions. Effects of addition of metal ions depended on the ions and on the media. Cadmium had negative impact on the linear growth rate whatever the medium used. A slighter effect was observed when adding lead or zinc.

Entomopathogenic fungi found on *Ips typographus* in Georgia

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Abstract: The spruce bark beetle, *Ips typographus* L. (Coleoptera: Scolytidae) is very common throughout Borjomi gorge forests in Georgia and causes considerable damage in stands of spruce trees (*Picea orientalis*). During a survey performed in 2000-2004 in several Georgian forests, the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium* sp. have been found on this insect.

Researches on entomopathogenic bacteria and applications for fly pest control in a Mediterranean region (Sardinia, Italy)

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Abstract: During the last few years, investigations were carried out in Sardinia (Italy) to isolate naturally-occurring soil bacteria and detect their toxicity against medfly (*Ceratitis capitata*), olive fruit fly (*Bactrocera olea*) and housefly (*Musca domestica*) as well as some of their pupal parasitoids. Strains of *Bacillus thuringiensis* were isolated and then comparatively studied with strains of other geographic origin. Morphological (presence of parasporal inclusions) and genetic (16S rRNA gene sequence, detection of *cry* genes) observations and protein profile analysis by SDS-PAGE were used for identification and characterization of the *Bacillus thuringiensis* isolates. Among about 300 different bacterial isolates, 4 were significantly toxic to housefly adults, 1 to housefly larvae, 11 to adult of olive fruit fly, 20 to olive fruit fly larvae, 4 to adult of medfly, and 22 to medfly larvae. No significant toxicity was detected against the Hymenoptera *Opius concolor* and *Muscidifurax raptor* (olive fruit fly and housefly pupal parasitoids, respectively). Experimental treatments in olive crops against olive fruit fly adults and in livestock against both housefly adults and larvae were carried out with formulations of the most toxic bacterial strains, encouraging the introduction of microbiological control in the integrated pest management of these fly species.

Microbiological control of lepidopterous defoliators in Sardinian cork oak forests

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Abstract: Mediterranean oak forests are exposed periodically to infestations of gypsy moth (*Lymantria dispar*). This insect, alone or in association with other lepidoptera, such as *Malacosoma neustria* and *Tortrix viridana*, can cause the total defoliation of thousands of hectares in one year. From 1990 to 1995, aerial spray trials using several preparations of *Bacillus thuringiensis* var. *kurstaki* against gypsy moth were performed in Sardinian cork oak forests to determine the efficacy of the preparations and the most suitable modalities for a Mediterranean environment. Based on these preliminary trials, a microbiological control program to limit the damage caused by *L. dispar* and *M. neustria* was implemented in approximately 45000 hectares from 2001 to 2004.

Comparison of the efficacy of AdorGV and chemical insecticides against the Summer fruit tortrix, *Adoxophyes orana*, in commercial apple orchards in the Czech Republic

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Abstract: The field trials of the Summer fruit tortrix (*Adoxophyes orana*) control in three commercial apple orchards were carried out in the Czech Republic in 2002-2004. *Adoxophyes orana* granulovirus, AdorGV (CAPEX 2), etofenprox (TREBON 10 F), flufenoxurone (CASCADE 5 EC), fenoxycarb (INSEGAR WP), triflumurone (ALSYSTIN 480 SC), triflubenzurone (NOMOLT 15 SC), phosalone (ZOLONE 35 EC), deltamethrine (DECIS EW 50) or acetamiprid (MOSPILAN 20 SP) were applied against the overwintering larvae or larvae of the first generation of the pest. From the tested insecticides, CASCADE 5 EC was the most effective against *A. orana*. CAPEX 2 had similar efficacy as CASCADE 5 EC except the first years of application. Efficacy of the other tested insecticides was insufficient against *A. orana*. When CAPEX 2 is used first time in locality, it is necessary to apply it 2-times against the overwintering larvae and then to make 2 applications against the larvae of the first generation (all in interval 7-10 days). The sequence of CAPEX 2 application given above reduces the population density of the 2nd generation of *A. orana* and in the 2nd year of treatment it prevents completely the fruit damage.

Evaluation of efficacy of *Adoxophyes orana* granulovirus on the reduction of *Adoxophyes orana* populations using PCR

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Abstract: The presence of *Adoxophyes orana* granulovirus (AdorGV) in larvae of the summer fruit tortrix (*Adoxophyes orana*) after treatment by AdorGV-based preparation Capex® 2 against *A. orana* was investigated in three localities in the Czech Republic in 2003-2005. A PCR assay was developed for this purpose; it could be used to identify the frequency of AdorGV in the insect populations infected with AdorGV as well as in natural populations. The AdorGV was detected in larvae of *A. orana* from all three localities treated by Capex® 2. The portion of the overwintering larvae positive for AdorGV differed according to locality and ranged from 5% to 80% after the treatment. The AdorGV was detected from 10% to 80% in larvae from localities, where AdorGV was disseminated by special pheromone traps. No AdorGV was detected in larvae collected in the locality without virus treatment. The results suggested a strong persistence of AdorGV in surviving larvae after direct treatment by AdorGV causing high mortality of larvae in next generations. The population density was reduced by AdorGV under damage threshold during two years after the virus treatment.

Codling moth granulovirus: First indication of variations in the susceptibility of local codling moth populations

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Abstract: This study is part of a BMVEL (German Federal Ministry for Consumer Protection, Food and Agriculture) project for prevention of codling moth damage by long-term population control on large areas. Local codling moth populations were collected in three different orchards in the South of Germany; two of them having been treated with CpGV over many years (Lake Constance II and South Baden) and one since two years (Lake Constance I). The susceptibility of the offsprings of the overwintering larvae for the granulovirus of codling moth (CpGV “Mexican strain”) was investigated in bioassays on artificial diet and compared to a laboratory strain of the codling moth. The results indicated significant differences in sensitivity to the virus between the three local codling moth populations. The determination of the LC₅₀-values showed that South Baden and Lake Constance II were more than thousand fold less susceptible than Lake Constance I and the laboratory strain. The slope of the dose-mortality-regression lines of populations South Baden and Lake Constance II was significantly lower than those of Lake Constance I and the laboratory strain. This indicates a high inhomogeneity in the individual response of the larvae against the virus. At present, it is not possible to judge the significance of these first observations for the practical use of the virus in the field. Therefore, more investigations of other local codling moth populations have been initiated.

Controlling wireworms (*Agriotes* spp.) in a potato crop with biologicals

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Abstract: Wireworms (*Agriotes* spp.), larvae of click beetles, are a serious worldwide soil pest mainly in potatoes (*Solanum tuberosum* L.), causing damages in summer and early autumn on the daughter tubers. It is an increasing problem in many European countries, like the U.K., Austria, Switzerland, the Netherlands and Italy. Damages consist of round holes on the surface and tunnels running into the tubers. This damage reduces yield but mainly quality of the crop even making a batch unmarketable. Field experiments were carried out in 2001 and 2002, to assess the protection against wireworm in potatoes of different compounds. Treatments were applied as a furrow or broadcast application or as a tuber drench. Compounds used in the trials were biologicals, entomopathogenic fungi and entomoparasitic nematodes or combinations with an insecticide. These compounds were compared with insecticide granulates of ethoprophos applied as a broadcast application at seedbed preparation, or with chlorpyrifos as a furrow treatment at the moment of planting and incorporated.

Assessments focused on the effects of the compounds on the emergence and number of stems per plant, the damage to the mother tubers and the damage to the daughter tubers at harvest. Phytotoxicity was observed from furrow treatments with carvacrol. Furrow applications with carvacrol or cinnamaldehyde or *Steinernema feltiae* were ineffective at controlling wireworms. *Beauveria bassiana* or the combined application of *B. bassiana* (furrow application) with imidacloprid (tuber drench) showed a significant reduction of wireworm damage in potatoes, this was comparable to the etroprophos granulates.

The peach flatheaded rootborer, *Capnodis tenebrionis* (L.), and its enemies

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Abstract: *Capnodis tenebrionis* (L.) (Coleoptera Buprestidae) is an important and common phytophagous insect of Mediterranean stone-fruit orchards. Current knowledge on its enemies is really scanty; consequently no biological control strategy has been set up. For this reason, a survey was carried out in South Italy areas. Only a bethylid species, *Sclerodermus cereicollis* Kieffer, and some entomopathogenic fungi were found, so confirming the extreme scarcity of *Capnodis* natural enemies. The susceptibility of the peachborer adults to 2 commercial formulations of *Bacillus thuringiensis* (Berliner) was assessed in laboratory bioassays. Both products proved to be totally inefficacious in controlling the beetles.

Viability of entomopathogenic microorganisms encapsulated in alginate pellets

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Abstract: Several authors have studied the applicability of sodium alginate formulations for different purposes. This kind of formulation seems to be quite suitable to control phytophagous insects living in the soil for at least one phase of their life cycle. Therefore, we devised a method to encapsulate entomopathogenic microorganisms in alginate pellets and evaluated the duration of their viability. Twenty bacterial and twenty-nine fungal isolates from agricultural and forest soils and from insect samples collected in several Italian localities were tested. In the viability tests, we periodically plated alginate pellets and checked the development of colonies on the growth media. The bacterial isolates maintained their viability for five years while the fungal isolates showed shorter survival, with a mean viability of 12-18 months. The results for bacteria were highly positive. However, for fungi, it would be necessary to modify the alginate formulation process to extend the storage period.

Occurrence of pathogens and parasites in *Ips typographus* L. from spruce stands (*Picea orientalis* L.) in Georgia

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Abstract: Occurrence of pathogens was investigated in the spruce bark beetle *Ips typographus* from two different sampling plots in Borjomi region at 900m above sea level, collected in September 2004. Investigations brought evidence of one pathogen species only, *Gregarina typographi*, was found in the mid-gut lumen of 48.6% and of 23.2% of adult beetles respectively. In both cases were more male beetles infected than female beetles. Furthermore, unidentified fungal infections were observed in some few individuals. Relatively few beetles had larvae of the Hymenopteran parasitoids *Tomicobia seitneri* and *Ropalophorus clavicornis*. In addition, *Contortylenchus typographi* nematodes were found in the haemolymph of beetles, and not identified nematode larvae in the gut lumen.

Survival of the spruce bark beetles, *Ips typographus*, infected with pathogens or parasites

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Abstract: The survival of adult *Ips typographus* beetles infected with four pathogens was tested under laboratory conditions, as well as the type and amount of the food offered to adult bark beetles necessary for optimum infections. Adult beetles were successfully infected with microsporidia, gregarines and nematodes, but viruses.

Occurrence of *Gregarina typographi* (Sporozoa: Gregarinidae) and *Metschnikowia cf. typographi* (Ascomycota: Metschnikowiaceae) in *Ips sexdentatus* (Coleoptera: Scolytidae) from Austria

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Abstract: *Ips sexdentatus* from seven Austrian locations were dissected and inspected with regard to the occurrence of pathogens. *Gregarina typographi* was found in specimens from all seven locations, *Metschnikowia cf. typographi* was found in the beetles from six locations. Prevalence of both pathogen species varied in the beetles from the different locations.

Taking biocontrol from laboratory into practice: a case study from Kenya

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Abstract: Dudutech is the largest IPM/biocontrol company in Eastern Africa and the only one presently developing microbial pesticides. The first products it successfully developed and registered were natural enemies, which are currently used commercially on a large scale both in vegetable and flower crops; however, the company is presently striving to develop several biopesticides and make them into a commercial reality. Amongst the microbials, the fungi, *Trichoderma asperellum* (for control of soilborne diseases) and *Pochonia chlamydosporia* (against *Meloidogyne* spp.), are already in commercial or semi-commercial production, together with the nematode, *Steinernema feltiae*. Other products in the pipeline include *Pasteuria penetrans* and a granulosis virus (*PlxyGV*), as well as some entomopathogenic fungi. Based on Dudutech's experience, the main factors affecting the development of biopesticides and their successful implementation on commercial crops in Kenya will be briefly discussed.