IOBC / WPRS

Working group „Insect Pathogens and Insect Parasitic Nematodes”

Subgroup “Soil Insect Pests”

OILB / SROP

Groupe du travail “Entomopathogènes et Nématodes Parasites d’Insectes”

Sous-Groupe “ Soil Insect Pests ”

PROCEEDINGS of the MEETING
COMPTES RENDUS de la REUNION

at / à

Auer/Ora (Italy)

16-18 October 2006

Editor:

Jürg Enkerli

IOBC wprs Bulletin

Bulletin OILB srop Vol. 30(7) 2007

Contents

Biology of *Hyalesthes obsoletus* and approaches to control this soilborne vector of Bois noir disease

M. Maixner
BBA, Institute for Plant Protection in Viticulture, D-54470 Bernkastel-Kues, Germany -M.Maixner@BBA.de

Abstract: The planthopper *Hyalesthes obsoletus* is the vector of Bois noir disease of grapevine and diseases of other plants associated with stolbur phytoplasma. The polyphagous vector acquires the pathogen while feeding on the roots of herbaceous reservoir plants. Occasional feeding of adults on grapevine causes infection and subsequent development of Bois noir symptoms. *H. obsoletus* is a xerothermic species that prefers sparse vegetation on open soil. The herbaceous host plants considerably influence distribution and density of *H. obsoletus* as well as the levels of infestation of vector populations. Since specific strains of the Bois noir phytoplasma occur in different host plants and the vector populations are adapted to their particular hosts, too, there are different epidemic cycles of the disease in the field. Control strategies for *H. obsoletus* focus on cultural practices like soil cultivation, weed control and green cover.

Management and biological control of wireworm populations in Europe: current possibilities and future perspectives

Lorenzo Furlan
Department of Agronomy, Entomology, University of Padova, Agripolis, via Romea 16,Legnaro PD; Italy E-mail: lorenzo.furlan@inwind.it

Abstract: To achieve a rational protection of sensitive crops from wireworm attacks in the framework of biological control, an overall strategy, taking into consideration all the information available, should be implemented. The strategy should be divided into 2 phases: 1) precise *Agriotes* population monitoring and damage prediction; 2) *Agriotes* population management which should be divided into: A) *agronomic strategies*: altering rotation, tillage timing, irrigation timing according to the life cycle of each *Agriotes* species, etc.; B) application of biological tools. The most effective biological control strategy is planting the sensitive crops where no high wireworm populations are present. Currently, it is possible to predict wireworm population levels with reliable results and low costs using the pheromone traps suitable for monitoring all the most important *Agriotes* species in Europe and then select the fields without any risk of wireworm damage to plant sensitive crops. When planting of a sensitive crop has to be done in fields with high wireworm populations regardless the risk for plant stand, the available tools on the market considered active against wireworms are limited. An assay carried out in 2006 to evaluate products available in the Italian market showed that currently only the biocidal defatted seed meals have a potential to reduce wireworm populations and to protect the crop. Biofumigation caused by the biocidal defatted seed meals proved to be as effective as the chemical treatment.

Occurrence of click beetle pest spp. (Coleoptera, Elateridae) in Europe as detected by pheromone traps: survey results of 1998-2006

Lorenzo Furlan[1], Miklos Toth[2]
[1]*Department of Agronomy, Entomology, University of Padova, Agripolis, via Romea 16,Legnaro PD; Italy;*
[2] *Plant Protection Institute, HAS, Budapest, Herman O. u. 15, H-1022Hungary*

Abstract: The implementation of IPM strategies against wireworms has been very difficult because of the shortage of reliable information on the key aspects of the species until few years ago. One of these key aspects is represented by the species distribution over the different European regions. Knowing the species present allows us to establish promptly oriented monitoring programs saving time and materials and a general prediction of damage risk for the sensitive crops based on the knowledge of biology and behaviour of the different *Agriotes* species. A reliable description of the distribution of the main *Agriotes* species is currently possible because pheromone traps suitable for monitoring all of the most important *Agriotes* species in Europe are available. They proved to be effective to detect the presence of species also at very low population levels. The maps of the distribution of the main *Agriotes* species in Europe are presented and commented.

Spatial distribution of click-beetles (*Agriotes* spp.) at field and landscape scales

Rod P. Blackshaw[1], Robert S. Vernon[2] , Helen Hicks[1]
[1] *School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA,UK`;*
[2]*Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Agassiz Research Station, P.O. Box 1000 – 6947, #7 Highway, Agassiz, British Columbia V0M 1AO, Canada. E-mail: rblackshaw@plymouth.ac.uk*

Abstract: Pheromone traps were used to study the spatial distribution of the click-beetles *Agriotes lineatus* and *A. obscurus* in the closed agricultural landscape of Westham Island, British Columbia, Canada over a three year period. Spatially referenced counts were analysed using Spatial Analysis by Distance IndicEs (SADIE). This showed that there were statistically significant patches of higher, and gaps of lower, counts. SADIE association analyses were applied and it was concluded that there was considerable temporal stability for both species at this scale, both between and within years, and that both species had similar spatial patterns. This study was extended to the smaller field scale at two sites in British Columbia, Canada. There was considerably less evidence for spatial stability and different patterns emerged for each species. The observed distribution patterns were dependent on the distance between pheromone traps and it was postulated that *A. lineatus* either moved faster, or was more responsive to pheromones, than *A. obscurus*. This hypothesis was tested in a mark release recapture field trial in England and was accepted. It is concluded that simultaneous counts of different species of click-beetles in pheromone traps cannot be uniformly interpreted.

European wireworms (*Agriotes* spp.) in North America: toxicity and repellency of novel insecticides in the laboratory and field

Robert S. Vernon, Wim Van Herk, Chandra Moffat, Chantelle Harding
Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, P.O. Box 1000, Agassiz, British Columbia, CANADA V0M 1A0.

Abstract: Three species of wireworms, notably *Agriotes obscurus*, *A. lineatus*, and *A. sputator*, were introduced to North America from Europe about a century ago. All three species are present in the Atlantic provinces of Eastern Canada, and *A. obscurus* and *A. lineatus* are present in the westernmost province of British Columbia, and in the states of Washington and Oregon in the USA. These species and several other endemic species have become major pests of a variety of crops across Canada and the USA, with their major impact being on potatoes, corn and cereals, Of major concern in Canada is that all of the most commonly used insecticides for wireworm control are no longer available, and attempts to register new and effective wireworm insecticides are proving to be difficult from both regulatory and biological points of view. Due to the complex subterranean activities of wireworms, and the time and expense required for wireworm sampling, contemporary efficacy studies often rely solely on crop stand, yield and/or cosmetic injury criteria when evaluating new insecticides. By extrapolation, circumstantial evidence of wireworm damage is often equated with reductions in wireworm populations. Although this assumption was likely correct with the older organochlorine and organophosphate chemistries (we have evidence for this), we have found that this does not apply to many of the newer chemistries being studied and registered for wireworm control. Studies in the laboratory and field, for example, have shown that although exposure to various neonicotinoids (i.e. thiamethoxam, clothianidin and imidacloprid) will suppress damage (i.e. corn, wheat) by several economic species of wireworms, this is due primarily to wireworms entering a rapid and long-term state of intoxication rather than mortality. Exposed populations recover later on in the growing season. In contrast, fipronil (a phenyl pyrazol) applied at higher dosages to wheat and potato crops resulted in excellent crop protection and virtual extermination of *A. obscurus* populations by the following growing season. We also found that applications of fipronil at low dosages to *A. obscurus* in the laboratory did not affect wireworm health immediately, but significant latent morbidity and mortality (up to 90%) began occurring after about 40 days. In specially-developed soil bioassays, we have also found that tefluthrin (a synthetic pyrethroid), although registered in Canada for wireworm control in corn, is actually repellent to *A. obscurus* and *Limonius canus*. Damage control with tefluthrin in corn, therefore, is likely due to wireworms being repelled rather than killed during the critical establishment stage. This paper discusses the implications of these data as they relate to the current and future screening of novel lower risk insecticides.

New approaches to wireworm management in the UK

William E Parker
ADAS, Woodthorne, Wolverhampton, WV6 8TQ, UK.

Abstract: Wireworms, particularly *Agriotes obscurus*, *A. sputator* and *A. lineatus* are becoming an increasing problem for UK farmers, particularly for growers of high value crops such as potato. The lack of effective insecticides for wireworm control means that alternative methods of control are needed to ensure that the risk of wireworm damage is minimised. In the UK, a new project on sustainable long-term management of wireworms is just starting. This will include work on novel approaches to control such as the use of biofumigants (*Eruca sativa* and *Brassica juncea*), and novel biocontrol agents such as *Metarhizium anisopliae*. Work will also be done investigating the interactions between cultivation and insecticide use on the maintenance/decline of wireworm populations in all-arable (no grass) rotations – a particular issue in the UK at present.

Comparison of three different bait trap types for wireworms (Coleoptera: Elateridae) in arable crops

Nina Brunner, Eva-Maria Grünbacher, Bernhard Kromp
Bio Forschung Austria, Rinnböckstraße 15 1110 Wien, Austria

Abstract: Wireworms are abundant dwellers of arable soils, causing economically severe damage on potatoes, maize and other arable crops (e.g. sunflower, rape, asparagus, lettuce, onion). Since pesticide application for direct control is not allowed in organic farming, assessment systems for detecting wireworm infestation levels and forecasting damage thresholds are urgently needed. However, the basis of any wireworm risk assessment is a proper sampling technique for recording wireworm occurrence and abundance in the soil. In an earlier study, comparing different baited and unbaited wireworm sampling methods, cereal baited traps were found to be most effective. The present study was carried out from May to September 2005 in two potato fields and one maize field in Vienna/Austria. It aimed at finding out the most effective and time-efficient method among three different cereal (maize/wheat mixture) baited trap types: I. plate-trap (Jossi & Bigler 1997), II. pot-trap (adapted from Kirfman & Armon 1986), III. mesh-bag (Horton & Landolt 2002, adapted by Brunner et al. 2005). In each field, 20 traps of each type (reduced to 6 traps from July onwards) were set along two transects (distance between sampling points: 7 m). Additionally, at each sampling point, one potato plant was inspected for wireworm damage before harvest. The traps were changed every 2 to 4 weeks. A soil core of 15 cm in diameter was taken around the traps to get hold of wireworms outside the containers. The bait mixture and the surrounding soil were hand-sorted separately for larvae. Altogether, 1.988 wireworms were trapped. Considering the total wireworms caught inside and outside the bait traps, the three trap types did not differ significantly from each other (Kruskal-Wallis-Test). However, considering only the larvae caught inside the traps, pot-traps contained significantly more larvae (3.3 inds/trap) than plate-traps (2.4 inds/trap; $p = 0.038$) and mesh-bags (1.2 inds/trap; $p < 0.001$; U-Test). As to the practical implementation in wireworm field-studies, we consider the pottrapping method advantageous, since 75% of baited larvae were found inside the pots, compared to 63% inside the plates and only 53% inside the mesh-bags. Therefore, bait-trapping with the pot method can be done without the time-consuming evaluation of the surrounding soil cores.

Practical Dutch experience introducing a monitoring system of click beetles by pheromone traps.

Klaas van Rozen[1], Albert Ester[1], Ton Hendrickx[2]
[1] *Applied Plant Research (PPO-AGV) Wageningen University and Research, P.O.Box 430,8200 AK Lelystad, The Netherlands.*
[2] *Coöperatieve Zuidelijke Aan- en Verkoopvereniging u.a. (CZAV), P.O. Box 402, 4460 AV Goes, The Netherlands*

Abstract: A new supervised control system against wireworms is introduced in the Dutch agriculture, using a click beetle trap (Kniptorkit) for monitoring the adult beetles, followed by a pyrethroid control at a defined time point if necessary. Therefore, the beetles instead of the wireworms are controlled. The Kniptorkit is a trap equipped with a sexual lure (pheromone), attracting male click beetles. Attractively for farmers who have already experience with wireworm problems on certain fields. Practical experience started in 2004 with 56 growers using this trap mainly in grass seed and wheat crops having potatoes in their crop rotation. In 2005 the number of growers increased to 105 participants using this monitoring and control system. Captures between regions and between several fields can differ strongly. Differences are caused by natural presence in the area, field history and the host plants for egg deposition in which the traps are placed. Click beetles deposit eggs mainly in monocotyle crops. It is important to observe the presence of the click beetles per field, since research has shown that wireworms are not found on every field. This may limit the use of pesticides, an initial aim next to provide growers good alternative controlling wireworms.

New sex attractant for *Agriotes proximus*: similarities in pheromonal communication with *A. lineatus* (Coleoptera: Elateridae)

Miklós Tóth[1] Lorenzo Furlan[2], Amália Xavier[3], József Vuts[1], Mitko Subchev[4], Teodora Toshova[4], István Szarukán[5], Venyamin Yatsynin[6]

[1] *Plant Protection Institute, HAS, Budapest, Herman O. u. 15, H-1022 Hungary;*

[2] *Department of Agronomy, Entomology, Padova University, Agripolis, Via Romea 16, Legnaro, I-35020 Italy;*

[3] *DRAEDM, Porto, Rua da Resauracao 336, P-4050-501 Portugal;*

[4] *Zoology Institute, Bulgarian Academy of Science, Sofia, blvd Car Osvoboditel 1, BG-1000, Bulgaria;*

[5] *Agricultural University, Debrecen, POB 58, H-4001, Hungary;* [6] *Krasnodarskiy NIISKh im. P.P. Lukyanenko, Krasnodar 12, Russia, 350012*

Abstract: When testing traps baited with a blend of geranyl octanoate and geranyl butanoate (the pheromone components of *Agriotes lineatus*, Coleoptera, Elateridae) in Portugal, large numbers of the closely related *A. proximus* were captured. This was highly surprising, as in the literature two completely different components, (*E,E*)-farnesyl acetate and neryl isovalerate were previously described as pheromone components of *A. proximus*. Later tests conducted in several countries of Western and Central Europe revealed that the butanoate on its own showed low but significant attraction for *A. proximus*, while the same was true for the octanoate and for *A. lineatus*. However, largest catches were observed with a blend of the optimal 1:1 ratio in both species. No *A. proximus* catches were recorded in traps baited with the blend of (*E,E*)-farnesyl acetate and neryl isovalerate at any of the test sites. In electroantennography (EAG), male antennae of both *A. proximus* and *A. lineatus* responded better to geranyl butanoate than to geranyl octanoate, suggesting that there were no differences between the two species at the sensory level either. In conclusion, we found remarkable similarities in the two species as far as pheromone perception (EAG responses) and field responses to pheromone components were concerned. As for pheromone composition when extracted from females, in *A. lineatus* the major component was found to be geranyl octanoate by earlier studies, while geranyl butanoate was detected only in traces. Pheromone extraction of *A. proximus* populations is planned in the future. The two species are considered clearly separated due to mainly one constant morphological difference but there is no study demonstrating biological separation. Genetic or classical biological studies would be needed to clear up this issue. From the practical viewpoint, it appears that the 1:1 blend of geranyl octanoate and geranyl butanoate can be used as a bait in pheromone traps for the detection and monitoring of both *A. lineatus* and *A. proximus* in Europe.

Approaches to wireworm control in organic potato production

Daniel Neuhoff[1], Christiana Christen[2], Andreas Paffrath[2], Ute Schepl[2]
1 Institute of Organic Agriculture, University of Bonn, Katzenburgweg 3, D-53115 Bonn, Germany;
[2] *Agricultural Chamber NRW, Dep. Ecological Agriculture and Horticulture, Gartenstr. 11, D -50765 Köln-Auweiler,Germany*

Abstract: Tuber damages caused by wireworms can result in significant economic losses in organic potato production. Since no synthetic insecticides are allowed in Organic Agriculture, wireworm control has to be managed by agronomic approaches. In 2005 organic potato field trials were carried out on 3 sites in Southern North Rhine-Westphalia, targeted at assessing the impact of variety choice (2 - 6 varieties) and harvest date (3 - 5 dates) on wireworm damage of potato tubers. Tuber yield and quality were recorded and submitted to ANOVA. Variety choice had a significant effect on tuber losses caused by wireworms. At the Auweiler site the highest losses were noted in cv. *Princess* (27.0%) and the lowest in *Nicola* (12.7%). Bringing forward tuber lifting dates to the middle of August resulted in significantly lower wireworm induced tuber losses compared with middle of September. Early harvesting can be recommended if tuber skin is sufficiently suberised and if cooling facilities are available for the tubers.

Promise versus performance: working toward the use of *Metarhizium anisopliae* as a biological control for wireworms

Todd Kabaluk[1], Mark Goettel[2], Jerry Ericsson[1], Martin Erlandson[3], Ffion Cassidy[1], Bob Vernon[1], Stefan Jaronski[4], Kenna Mackenzie[5], Lee Cosgrove[6]
Agriculture and Agri-Food Canada: [1]*Pacific Agri-Food Research Centre, Box 1000, Agassiz, British Columbia, Canada V0M 1A0;*
[2]*Lethbridge Research Centre, Alberta;*
[3]*Saskatoon Research Centre, Saskatchewan;*
[4]*United States Department of Agriculture, Sidney, Montana;*
[5]*Altantic Food and Horticulture Research Centre, Kentville, Nova Scotia;*
[6]*address unknown*

Abstract: A local isolate of *Metarhizium anisopliae* has shown promise as a biological control for wireworms based on repeated success in laboratory and greenhouse studies. Several years of field data have shown mixed results when *M. anisopliae* has been used by a variety of application methods. Laboratory experiments designed to explain these mixed results have pointed to certain biotic and abiotic factors as influential factors. In an attempt to improve efficacy, *M. anisopliae* strain F52 (also know as BIO1020, BIPESCO-5) was field-tested in 2006 in combination with the insecticides clothianidin and spinosad in potato and corn, and improved yield was found in corn. *M. anisopliae* isolate characteristics, including growth and sporulation, productivity using solid state fermentation, compatibility with agri-chemicals and pathogenicity toward several species of the European and North American wireworms are presented.

Evaluation of *Metarhizium anisopliae* isolates for biocontrol of *Agriotes* based on genetic, biochemical and virulence characters

Vandana Ghormade[1], Werner Jossi[1], Santosh Chavan[2], Arumugam Rajendran[2], Amey Ghondhelekar[2], Franco Widmer[1], Siegfried Keller[1], Jürg Enkerli[1]
[1]*Molecular Ecology, Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH 8046 Zürich, Switzerland;*
[2]*Biochemical Sciences Division, National Chemical Laboratory, Pune 411008, Maharashtra, India.*

Abstract: The fungus *Metarhizium anisopliae* naturally infects *Agriotes* spp. and it has a great potential for use as a BCA against this pest insect. Fungal strains that are evaluated for biocontrol typically are selected based on their virulence towards the target host, which is assessed with bioassays. However, in the case of *Agriotes,* bioassays are very laborious and time consuming. Our goal was to assess alternative criteria i.e. biochemical and genetic strain characteristics as well as virulence assessment on the alternative host *Helicoverpa armigera* for pre-selection of *M. anisopliae* strains suitable for *Agriotes* control. A collection of 22 *M. anisopliae* isolates was used to investigate and compare the various characteristics. Genetic characterization was based on microsatellite markers as well as on PCR-RFLP analyses of two genes coding for proteases and three genes coding chitinases and biochemical characterization was based on protease and chitinase enzyme activities. The virulence on *H armigera* was determined for all 22 isolates however, virulence on *Agriotes* spp. was determined only for a subset of 7 isolates due to a limited number of *Agriotes* spp. larvae available. Assessment of correlations between all the different characteristics revealed significant correlations between chitinase activity and virulence on both hosts, indicating that chitinase activity may be used as an alternative criterion for pre-selection of *M. anisopliae* strains for *Agriotes* control. There was no significant correlation between virulence on *H. armigera* and virulence on *Agriotes*. However, bioassays with *H. armigera* allowed for identification and elimination of strains with low virulence. Therefore, bioassay with *H. armigera* might be a suitable tool for the pre-selection of virulent *M. anisopliae* strains. The number of isolates investigated and compared in this study was low and therefore conclusions have to be considered preliminary.

Investigations on click beetles using pheromone traps

Muhammad Sufyan[1], Daniel Neuhoff[1], Lorenzo Furlan[2]
[1] *Institute of Organic Agriculture, University of Bonn, Katzenburgweg 3, D-53115 Bonn, Germany;*
[2] *Dep. of Agronomy, Entomology, University of Padua, Agripolis, via Romea 16, Legnaro PD, Italy.*

Abstract: Wireworms may cause damages in organic crops such as potatoes, carrots and maize. Since no synthetic pesticides are allowed in Organic Agriculture, control strategies need to be targeted on agronomic approaches and on biological tools interfering with the population dynamics. In North Rhine - Westphalia / Germany several strategies were investigated at the Institute of Organic Agriculture / University of Bonn, including an evaluation of mass trapping/sex disruption by using pheromone traps for *Agriotes lineatus*, *A. obscurus* and an assessment of the range of attractiveness of the pheromone traps used. Experiments on seasonal fluctuation of adult population between 2004 and 2006 indicated that the swarming period lasted from late April to end of August with one main peak in May and one weaker peak in mid June respectively. A tendency to decreased wireworm population was noted in plots with the permanent presence of sex pheromone traps and the continual removal of the males over three years compared with an untreated control area (no male trapping). Experiments focussed on estimating the range of attractiveness of the pheromone traps indicated that the recovery rate of the released beetles (*A. lineatus* and *A. obscurus*) to the traps was rather dependent on release distance than on time. Recovery rates for both species > 60% were only noted in clover grass for release distances up to 10m, while less than 10% of the beetles released at a distance of 60 m returned to the trap. Recovery rates of beetles released on bare soil were generally lower compared with clover grass. More than 50% of the males were recovered within the first 24 hours.

The swarming flight of Common cockchafer *Melolontha melolontha* L., 1758 (Coleoptera, Scarabaeidae) in two different areas of Bavaria and an approach to control the egg deposition

Ullrich Benker, Bernhard Leuprecht
Bavarian State Research Centre for Agriculture (LfL), Institute for Plant Protection, Lange Point 10, D-85354 Freising, Germany

Abstract: In Bavaria there are two well known large populations of the common cockchafer *Melolontha melolontha*. One of them is located in the Hessenthal-Mespelbrunn valley in the north-west of Bavaria (Spessart), where in the last four years the Institute for Plant Protection in Freising made several trials to control the grubs. The other population is located south of Augsburg in the rural district of Landsberg/Lech within and around the small village Reichling. In 2006 in both places the swarming flight of *M. melolontha* was expected and this was a good chance to observe the behavior of the beetles in these two completely different landscapes. In Hessenthal-Mespelbrunn cockchafers represent an important pest in the grassland and therefore, farmers need efficient control measures. The grubs feed on the roots of plants and in addition the sward is destroyed and turned upside down by wild pigs digging for the grubs. Pilot experiments to control *M. melolontha* were carried out with four insecticides with the active ingredients neem (Neem Azal), λ-cyhalothrin (Karate Zeon), dimethoat (Perfekthion) and *Bacillus thuringiensis* (Novodor) to evaluate the most effective treatment for possible future large scale applications. The most effective insecticide in this experiment was λ-cyhalothrin, followed by dimethoat. However, it is uncertain whether these insecticides will be applied in the future, because there are public concerns and objections from different organisations. Another approach was tested within the most threatened area in the Hessenthal-Mespelbrunn valley. The aim of this approach was to attract egg laying females to defined plots with short cut grass, in which after egg deposition and hatching of the larvae mechanical control as well as biological control measures in the form of *B. brongniartii* could be applied in the following spring. In the Lechgau area at Reichling the cockchafer *M. melolontha* is not considered an agricultural pest, although in 2006 the trees in Reichling were more seriously infested with beetles compared to the Spessart region. The more or less flat grassland in Reichling receives enough rainfall, the meadows are dominated by dandelion and the damage of the grubs in the grassland is negligible. Farmers in this area tolerate the grubs. The damage caused by the beetles nearly disappears with a second shoot of the foliage and garden owners also see no need for an extensive control of the cockchafers. In fact, quite the contrary was the case as tourists were coming from distances of about 100 kilometres to collect beetles.

Spraying adult forest cockchafers with *Beauveria brongniartii* conidiospores: preliminary results of a large field trial nearby Darmstadt during the main flight in 2006

Kerstin Jung
BBA, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany

Abstract: In the vicinity of Darmstadt, 100 ha of forest were treated with an experimental preparation of *Beauveria brongniartii*-conidiospores, applied by helicopter, during the main flight of the forest cockchafer, *Melolontha hippocastani* in 2006. Accompanying research comprised quality control of the experimental preparation, monitoring the fate of *B. brongniartii* after application, observation of the direct effects on beetles as well as monitoring of the effects on the next generation, both in the lab and in the field. Quality of the conidiospore powder was good in terms of purity, but it contained only half of the anticipated number of viable spores. Therefore, instead of 2x 10^{13} spores ha^{-1} only $3.8x10^{12}$ spores ha^{-1} were applied in total. Data on the effect of *Beauveria* on the cockchafer population collected in the field so far are incomplete and premature. However, in the area treated with *B. brongniartii* conidiospore powder, less first instar larvae were found (24 ± 19 m-2) compared to the untreated control area (38 ± 42 m-2). Conclusive results will be available in 2010 only, when the next beetle generation will emerge.

White grub control in golf courses

Siegfried Keller, Christian Schweizer
Agroscope Reckenholz-Tänikon Research Station ART, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

Abstract: A first white grub damage in a golf course in Switzerland was reported in the year 2000. Since then the number of severely damaged golf courses increased to six. In addition to that numerous damages were reported from sport and school areas as well as from airfields. Damages in golf courses are all located in alpine valleys and were done either by *Amphimallon solstitiale, A. majale, Phyllopertha horticola* or *Melolontha melolontha* or by a variable association of these species. Depending on the pest species present we applied *Metarhizium anisopliae* alone or in combination with *Beauveria brongniartii*. The application was done with a commercial drill machine, exceptionally with a rotary machine. The results achieved so far show that the fungi established in the soil and reduced of the white grub populations so that no more damages were visible.

Entomopathogenic nematodes and target soil insect pests in tree habitats in the Czech Republic, with focus on sawflies and cockchafers

Vladimír Půža [1,2], Zdeněk Mráček[2], Jaroslav Holuša[3]
[1]*Faculty of Biological Sciences, University of Southern Bohemia, Branisovska 31, Ceske Budejovice, Czech Republic;*
[2]*Institute of Entomology, BC AVCR, Branisovska 31, Ceske Budejovice, Czech Republic;*
[3]*Forestry and Game Management Research Institute Jiloviste-Strnady, Czech Republic*

Abstract: A long-term survey performed in the Czech Republic shows that EPNs naturally occur in the majority of tree habitats and have therefore a potential to control resident insect pests. One of the most important target insect pests in tree habitats are sawflies and cockchafers. A study focused on EPNs and web spinning larch sawfly (*Cephalcia lariciphila*) was started in the larch-spruce forest in the Czechomoravian highlands. Two sites – one with an outbreak of the sawfly and one control site with a sporadic incidence of *C. lariciphila* were chosen for the sampling. In spring and autumn 2005 and 2006 the sites were examined for the presence and abundance of EPNs and for the abundance and composition of the soil insect community. At the site with the sawfly outbreak a very abundant population of EPNs (approx. 30 000 infective juveniles per m2) was detected, while the population of EPNs at the control site was more than ten times lower. Both sites are inhabited by a variety of potential insect hosts of EPNs (mainly dipterans: Sciaridae, Dolichopodidae, Empididae, and coleopterans: Cantharidae, Carabidae) on which *S. kraussei* probably survive periods with low density of *C. lariciphila* larvae in the soil. Naturally nematode infected *Cephalcia* larvae and newly hatched adults were found during the study. Natural parasitisation attained 20 %. In spring 2006, a site with a serious outbreak of *M. hippocastani* near Lipnik, Cetral Bohemia, was thoroughly surveyed for EPNs. Four steinernematid species (*Steinernema kraussei*, *S. silvaticum*, *S. intermedium* and *S. feltiae*) were isolated from the area. These nematodes will be tested for pathogenicity towards *Melolontha* larvae.

A great increase of population of Common Cockhafer (*Melolontha melolontha* L.) in Idrija region in Slovenia

Anka Poženel, Mojca Rot
Agriculture and Forestry Institut Nova Goric, Pri hrastu 18, SI-5000 Nova Gorica, Slovenia

Abstract: During 2002 and 2006 a great increase of population of Common Cockhafer (*Melolontha melolontha* L.) was observed in the Idrija region in Slovenia. In 2002 the third larval stage of cockchafer with an average of 100 grubs per m2 completely damaged 370 ha of grasslands. In 2004 damage was caused by adult cockchafer. After egg deposition the population increased to 200 grubs per m2. Grass was damaged up to 50 % by the grubs of the first larval stage. In 2005 an average of 226 grubs per m2 was observed in the region. 760 ha of grasslands were damaged, which represents 62% of all agricultural land in the region. Different methods (mechanical, biological and chemical treatments) were used to reduce the population of the pest, but they were only partly successful. In June 2005 92 ha of grasslands were treated with *Beauveria brongniartii.* The efficiency of *Beauveria* was 38,7 %. The total decrease in the number of grubs in the treated area was 88 %.

Tipula paludosa population dynamics: challenging the myth of environmental limitation

Rod P. Blackshaw[1], Sergei V. Petrovskii[2]
[1]*School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK;*
[2]*Department of Mathematics, University of Leicester, Leicester, LE1 7RH, UK.*

Abstract: The larvae of *Tipula paludosa* – leatherjackets - are well known pests of grassland and cereals in northwest Europe and are also now becoming a problem in North America. It has become widely accepted that low rainfall at the time of egg and first instar reduces populations, and conversely, wet conditions at this time promote numbers. A meta-analysis of survey data shows there is substantial evidence for negative feedback between growth rate and population size irrespective of local conditions. This relationship is quantified and used

Challenges in using *Metarhizium anisopliae* for biocontrol of sugarbeet root maggot, *Tetanops myopaeformis*

Stefan T. Jaronski[1], Cynthia Fuller-Schaeffer[1], Kerstin Jung[2], Ayanava Majumdar[3] , Mark Boetel[3]
[1]*USDA ARS Northern Plains Agricultural Research Lab, Sidney MT;*
[2]*Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany;*
[3]*Department of Entomology, North Dakota State University, Fargo ND*

Abstract: *Metarhizium anisopliae* is under development as a microbial pest control agent of the Ulidiid fly, *Tetanops myopaeformis* (Sugarbeet Root Maggot), the most serious sugar beet pest in the United States. The fungus can be deployed by several means to create a "minefield" of infectious spores in the habitat of young larvae migrating to the developing root: (1) placing conidia on/in the seed coat to allow the fungus to colonize the rhizosphere; (2) applying *Metarhizium* granules around the seed at planting, much like insecticide granules; or (3) applying an aqueous spray of spores at or before peak fly oviposition to a narrow band of soil at the base of plants, allowing spores to soak into the top 1 cm of soil, where eggs are laid. A number of constraints could affect successful control by limiting *Metarhizium* survival before spores can contact larvae and influence conidial acquisition. We have examined several factors affecting *Metarhizium* performance: conidial and fungal granule concentrations in the soil; soil type (texture), moisture, and temperature influences on efficacy and persistence; the extent of rhizoplane/rhizosphere colonization; the effect of common planting-time fungicides; and interactions with a sample of rhizosphere-associated bacteria. The value of plantingtime granule and preovipositional conidial sprays in high and low insect pressure situations was also determined in replicated field trials.

Aggregation attractants for the sugar-beet weevils *Bothynoderes punctiventris* and *Conorrhynchus mendicus* (Coleoptera, Curculionidae, Cleoninae): application opportunities

Miklós Tóth[1], Lorenzo Furlan[2], Giovanni Campagna[3], Zoltán Imrei[1], Ivan Sivcev[4], Ivan Tomasev[5], István Ujváry[6]

[1]*Plant Protection Institute, Hungarian Academy of Science, Budapest, POB 102, H-1525, Hungary;*

[2] *Department of Agronomy, Entomology, Padova University, Agripolis, Via Romea 16, Legnaro, I-35020 Italy;*

[3] *COPROB, Minerbio, BO, Via Mora 56, I-40061 Italy;*

[4] *Institute for Plant Prot. & Environ., Belgrade, POB 33-79, SCG-11040 Rep. of Serbia and Crna Gora;*

[5] *Ministry of Agriculture and Forestry, Republic of Serbia, Phytosanitary Station for Plant Protection Subotica, Rep. of Serbia and Crna Gora;*

[6] *Institute of Biomolecular Chemistry, Chemical Research Center, Hungarian Academy of Science, Budapest, Pusztaszeri út 59/67, H-1025, Hungary*

Abstract: Earlier we discovered that a mixture of Grandlure I, II and III-IV showed attractancy for the sugar-beet weevil [*Bothynoderes (Cleonus) punctiventris*], which is a very important pest of sugarbeet in Eastern Europe. According to our most recent studies in Italy, the same compound mixture was attractive also for the closely related weevil *Conorrhynchus (Cleonus) mendicus,* which occurs in the western and southern part of Europe and in North Africa. Studies on the activity of the single components showed that only Grandlure III/IV [(*Z*)- and (*E*)-(Δ)3,3-dimethylcyclohexylidene acetaldehyde] was responsible for activity in both *B. punctiventris* and *C. mendicus*. Pitfall traps baited with our new attractant were ca 10 times more sensitive for *C. mendicus* than traps without this compound. Our present results suggest that Grandlure III/IV may be more widespread as an attractant in the subfamily Cleoninae than thought before. This is surprising, since this compound has been described so far from the pheromones of *Anthonomus* spp. (Curculioninae) and *Pissodes* spp. (Calandrinae). From a practical point of view it is of advantage that the two most important sugar-beet weevils in Europe can now be captured and monitored with the same synthetic attractant. Mass trapping efforts during two years on *B. punctiventris* showed that traps with this attractant at a density of 10 to 30 trap/ha were capable of trapping out a greater part of the population, thus the traps show perspective also for direct population control of sugar-beet weevils.

Metarhizium spp. against locusts and grasshoppers – a short review and future prospects

Barbara Pernfuss[1], Roberto Kron Morelli[2], Roland Zelger[3], Hermann Strasser[1]
[1] *Institute of Microbiology, Leopold-Franzens-University, Technikerstrasse 25, A-6020 Innsbruck, Austria*;
[2]*Agrifutur s.r.l., Via Campagnole 8, I-25020 Alfianello (Brescia), Italy.*
[3]*Landund Forstwirtschaft-liches Versuchszentrum Laimburg, Laimburg 6, I-39051 Pfatten/Auer, Italy*.

Abstract: A short review on the use of *Metarhizium anisopliae* var. *acridum* and *M. anisopliae* var. *anisopliae* as biocontrol agents (BCAs) against locusts and grasshoppers is presented. Mainly two projects, i.e. i) LUBILOSA (LUtte BIologique contre les LOcustes et SAutériaux) in Africa, and ii) CSIRO in Australia aimed to study the efficacy of BCAs against locust and grasshopper pests and developed products on the basis of *Metarhizium* spp., which were efficacious in the field and are accepted by the farmers. Currently, a plague of Siberian Grasshoppers (*Aeropus sibiricus*) devastates ecologically sensible alpine regions. Furthermore, mainly from South European Countries augmented reports are arriving which concern increasing crop ruination because of grasshoppers and locusts which accelerate possibly because of the global warming. This is why both the knowledge of the above mentioned projects and our own experience with biological control and *M. anisopliae* will be tied in order to develop an adequate application of *Metarhizium* spp. for the control of grasshopper and locust pests in Europe.

Persistence of GRANMET®, *a Metarhizium anisopliae* based product, in grape phylloxera-infested vineyards

Martin Kirchmair[1], Marc Hoffmann[2], Sigrid Neuhauser[1], Hermann Strasser[1], Lars Huber[2]
[1]*Leopold Franzens-University of Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria;*
[2]*Johannes Gutenberg-University of Mainz, Institute of Zoology, D-55099 Mainz, Germany E-mail: martin.kirchmair@uibk.ac.at*

Abstract: Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is one of the most serious grape pests worldwide. After successful bio-tests and pot experiments with the entomopathogenic fungus *Metharizium anisopliae* against grape phylloxera a field trial was started in spring 2003 in the German Rheingau to assess the efficacy of *M. anisopliae* against this aphid. In 2004 a second phylloxera infested vineyard was treated with GRANMET, Agrifutur s.r.l.. Three months after application an increase of the *Metarhizium* density in soil could be observed in both vineyards (>5 x 10^3 cfu g^{-1} dry wt soil). Compared with untreated plots a lower grape phylloxera-infestation could be observed in the *M. anisopliae*-treated plots. One year after treatment the maximum density of *Metarhizium* was estimated (1 x 10^4 cfu g^{-1} dry wt soil), accompanied with very low grape phylloxera infestations. Two years after treatment a control effect on grape phylloxera could still be observed whereas the density of the BCA in soil decreased. Three years after treatment no effect on the grape pest was detectable and the *Metarhizium* density in soil had decreased to a value similar to that in the control plots (<3 x 10^3 cfu g^{-1} dry wt soil). Therefore, a periodically application is necessary for an efficient control of grape phylloxera.

Are genetic algorithms a “magic bullet” for optimising cultivation conditions for entomopathogenic fungi?

Stefan Hutwimmer, Wolfgang Burgstaller, Hermann Strasser
Institute of Microbiology, Leopold-Franzens University Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

Abstract: In this article, basic considerations about the design of cultivation media for entomopathogenic fungi, its importance and its barriers are depicted. Genetic algorithms are proposed to improve this situation and their fundamentals and advantages are presented.

Assessment of virulence test-systems for quality assurance using sub-cultivated *Beauveria brongniartii* conidia

Angelika Loesch, Stefan Hutwimmer, Barbara Pernfuss, Hermann Strasser
Institute of Microbiology, Leopold-Franzens-University Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria

Abstract: Producers of biocontrol agents must describe and specify assay methods for product standardisation to ensure its purity and uniformity. Based on the approved and registered *Beauveria brongniartii* isolate BIPESCO 2, conidia from three different batches ((i) stored in liquid nitrogen; (ii) isolated from a mycosed *Melolontha melolontha* larva; (iii) harvested from MELOCONT®-Pilzgerste) were sub-cultivated up to ten times on four types of nutrient media. The harvested conidia were tested for their virulence against *M. melolontha* and *Tenebrio molitor* larvae using the BIPESCO standard operation method for bioassays. Conidia were also characterised for their carbon utilization profile using the BIOLOGTM SF-P2 and BIOLOGTM SF-N2 microtiter plates (128 carbon sources in total). The utilisation rates of carbohydrate compounds and -groups, respectively, were correlated to the virulence behaviour of sub-cultivated conidia with the aim to identify virulence markers. Furthermore, the radial growth of fungal colonies and their conidiation was assessed after each transfer. These assays confirmed that a successive transfer of *B. brongniartii* conidia *in vitro* is possible at least for five times without a demonstrable loss of virulence. The most relevant method for virulence control of *B. brongniartii* will be presented, which is a main issue for quality assurance of microbial pest control agents.