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Report on the 28th Annual Meeting of the Working Group "Beneficial Arthropods and Entomopathogenic Nematodes"

The 28th Annual Meeting of the Working Group "Beneficial Arthropods and Entomopathogenic Nematodes" of DPG and DGaaE was held on November 24–25, 2009, at the Gartenbauzentrum Schleswig-Holstein in Ellerhoop. The meeting was perfectly organized by Heike Rose and Tobias Plageman from the Landwirtschaftskammer Schleswig-Hostein and was attended by 49 participants from research institutions, universities, extension services and biocontrol companies. The scientific progamme comprised 25 contributions (20 presentations, four posters and one scientific movie), covering the following topics: (1) biocontrol of major agricultural pests, (2) impact of climate change on population dynamics of pests and beneficials, (3) invasive species and their control by beneficials, (4) role of beneficials in agroecosystems, (5) mode of action, selection, production and application of beneficials. The meeting started on the 24th early afternoon, continued during the "evening session" in a nice restaurant at Pinneberg nearby the venue and closed at noon of the following day. For the first time, it was held in combination with the "18th Workshop of Biological Control", where representatives of private and governmental extension services, biocontrol manufacturers and other practitioners of biological control in horticulture annually meet for networking and exchange of ideas, accompanied by short reports on practical work. About one third of our group also participated on this workshop which subsequently started on 25th. It was decided to repeat this attractive "double event" also in the following years. Our next meeting will take place from the 30th of November until 1st of December 2010 at the Julius Kühn-Institute Berlin-Dahlem. We would like to thank all contributors who submitted their abstracts for publication.

Dr. Annette Herz and Prof. Dr. Ralf-Udo Ehlers

Efficiency of entomopathogenic nematodes on the frit fly *Oscinella frit* under laboratory and field conditions

N. El-Wakeil[1,2], I. Bormann[2] & C. Volkmar[2]

1 Pests and Plant Protection Dept., National Research Center, Dokki, Cairo, Egypt; n_emara@islamway.net
2 Institute of Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Halle

Insect pests can cause great damage during the establishment phase of wheat because few tillers are present and many seedlings can be killed by a low incidence of pest individuals. Larvae of dipterous stem-borer frit fly *Oscinella frit* (L.) can cause considerable damage in newly sown summer wheat. Frit fly population was surveyed by using different colour water traps to determine the suitable date to control this pest. The efficiency of three types of entomopathogenic nematodes (EPNs) and one pyrethroid insecticide (Karate SC 9.4% (lambda -cyhalothrin)) were evaluated to control frit fly in the laboratory and also in the field on two wheat varieties (German (Triso) and Egyptian (Sakha 93)). Wheat seedlings of the German variety grew more rapidly than those of the Egyptian variety, causing differences concerning frit fly oviposition and infestation. The EPN *Heterorhabditis bacteriophora* had a higher efficiency in the laboratory than *Steinernema carpocapsae*, while the latter was better in the field. The numbers of frit flies caught in the water traps were recorded in two peaks on May 4 and 11, 2009. On May 4, 56, 49 and 34 flies were caught in the blue, white and yellow traps, respectively. The corresponding records on May 11 were 18, 39 and 16 flies. Populations of frit-fly larvae were higher in the untreated than treated plots. EPNs and Karate were sprayed on April 23 and on May 7. All treatments caused a reduction in frit fly infestation and increased larval mortality. The yield index was higher in the German than in the Egyptian variety in weight of grains/plot and yield/ha. Yield of all treated plots was higher than those of untreated plots. Karate and *S. carpocapsae* treatments resulted in significantly lower population densities of frit fly and significantly higher yields, followed by treatment with *S. feltiae*. The results indicated that the tested compounds were effective against frit fly. EPN may be used as biological control agents in IPM programmes.

Experience with *Amblyseius swirskii* in cucumber

M. Scharf

Pflanzenschutzdienst Hamburg, Hamburg;
michael.scharf@bwa.hamburg.de

The predatory mite *Amblyseius swirskii* feeds on eggs and primary larval stages of white flies, thrips and spider mites and on pollen. Trials were made by the Pflanzenschutzdienst Hamburg to show the impact of *A. swirskii* on pest infestation in cucumber. Potential effects on pest development were observed in one greenhouse compartment with *A. swirskii* releases in comparison to one without adding the predatory mite. About 4 weeks after transplanting, 100–120 *A. swiskii*/m^2 were released. *A. swirskii*, white flies and thrips were counted weekly on yellow and blue sticky traps and on leaves. The predatory mite established at high level on cucumber leaves for at least 3 months. White flies (*Trialeurodes vaporariorum*) were reduced twenty fold compared to the compartment without releases of *A. swirskii*. In both compartments the parasitic wasp *Encarsia formosa* was released in addition. *Frankliniella occidentalis* was the dominant species of thrips, which were reduced about tenfold in the compartement with *A. swirskii*-releases. In contrast, the effect on spider mite populations was minimal. According to our results, the application of *A. swirskii* in combination with the standard beneficials *Encarsia formosa* against white flies and with *Amblyseius cucumeris* against thrips can be recommended. In cucumber one preventive release will be sufficient.

Entomopathogenic nematodes for the control of diapausing larvae of the Codling moth (*Cydia pomonella* L.)

J. Kienzle[1], D. Heinisch[4], J. Kiefer[3], M. Trautmann[5], F. Volk[3], J. Zimmer[2] & C.P.W. Zebitz[6]

1 Kernen, Germany; jutta@jutta-kienzle.de
2 DLR Rheinpfalz, Rheinbach
3 Biofa AG, Münsingen
4 Bio-Obst GmbH Baderitz, Sornzig- Ablass
5 KOB Bavendorf, Ravensburg
6 University of Hohenheim, Institute of Phytomedicine, Stuttgart

The efficacy of entomopathogenic nematodes (EPN) against overwintering larvae of the codling moth (*Cydia pomonella* L.)

was reported by Lacey et al. (2006). In a project financed by the "Deutsche Bundesstiftung Umwelt" several methods of CM control were tested in order to check for their antagonistic potential and to further improve application. The potential of EPN as a tool for resistance management in codling moth control was examined. In a first approach, the hideout spots of overwintering codling moth larvae were identified. For this purpose, different part of the trees and the posts were removed and stored separately until hatching of the adults. Most larvae were found (1) in the bottom part of the stem, (2) in aerial roots in the upper part of the trees, (3) in bamboo posts cracked open, (4) in pinewood posts with vertical cracks. Fourteen large plot trials in different regions with application in autumn were assessed in summer of the following season. In two small plot trials trees were removed and hatching of adult moths was assessed in 2007/08 and 2008/09. The application of *Steinernema feltiae* with a rate of $0{,}75 \times 10^9$ per ha and meter tree height in most cases reduced the infestation in the field in the year following application. Efficacy was ranging between 40 to 50% when weather conditions were favourable. If the weather conditions were not favourable during a period of about 12 hours after application, efficacy of the treatment was decreasing. In Germany, except in Northern Germany, weather conditions are favourable for EPN application usually only during a few days in autumn. Since the organization of EPN application is easier to organize after harvest when temperatures are lower, the use of the low-temperature-active nematode species *Steinernema feltiae* is recommended, except for orchards with irrigation systems. Further research is done on the development of formulations reducing the risk of low efficacy during unfavourable weather conditions.

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Biology, genetic identification and effectiveness of biological control agents against *Otiorhynchus* spp.

J. Hirsch & A. Reineke

Geisenheim Research Center, Department of Phytomedicine, Geisenheim; hirsch@fa-gm.de

Several members of the weevil genus *Otiorhynchus* (Coleoptera: Curculionidae) are becoming increasingly important as pests in a wide variety of horticultural crops worldwide. Among them, the black vine weevil *Otiorhynchus sulcatus* is the most important and therefore best studied species. Besides *O. sulcatus*, a recent survey in Germany and other European countries has shown that several other *Otiorhynchus* species are present on diverse horticultural crops, causing more or less similar leaf notches like adult *O. sulcatus*. While the feeding on the foliage of their host plants by adult weevils can be regarded as a less significant damage, the feeding of soil-borne larvae on the roots can be lethal especially for recently transplanted cuttings or younger plants. Species identification of adult weevils is difficult but the morphological determination of *Otiorhynchus* larvae is almost impossible. The determination of the respective species, especially in the damaging larval stage, is a prerequisite for developing and applying efficient pest management strategies. We therefore developed a diagnostic PCR-RFLP method to identify 16 *Otiorhynchus* and 7 other weevil species, independently of their developmental stage. This molecular identification method is robust, cost-effective and provides reliable results within at most five hours after DNA isolation. We suggest using it in the future by plant protection services for diagnostic purposes. To evaluate future control options, we also assessed the efficiency of natural antagonists such as fungi or predators against some of the most common *Otiorhynchus* species. Among them, we tested the fungus *Beauveria bassiana* strain ATCC 74040 (Naturalis®) against adult weevils in bioassays. Our first results indicate that different *Otiorhynchus* species are differentially susceptible towards this fungus. Carabid beetles are regarded as effective natural antagonists against Coleopteran larvae, however, their potential against *Otiorhynchus* larvae is yet unknown. In first laboratory studies we demonstrated that *Harpalus* and *Nebria* ground beetle species feed on *Otiorhynchus* larvae. Field surveys in nurseries or market gardens have shown that both species are occurring in numbers high enough to cause a substantial reduction of the pest. Currently, we are developing a molecular method to detect weevil DNA in the gut or stomach of ground beetles in order to evaluate the influence of carabids against *Otiorhynchus* larvae in the field.

Use of the entomopathogenic nematode *Steinernema carpocapsae* against the adult stage of *Otiorhynchus sulcatus*

T. Ufer[1], A. Peters[2], A. Wrede[1]

[1] Chamber of Agriculture Schleswig-Holstein – Department for Horticulture, Ellerhoop-Thiensen; awrede@lksh.de
[2] e-nema GmbH, Schwentinental

The black vine weevil *Otiorhynchus sulcatus* is well known as a major pest in container grown woody ornamentals. Their biological control with entomopathogenic nematodes (EPN), e.g. *Heterorhabditis bacteriophora*, has been proven to be a suitable method in horticultural practice many years ago. However, the control of the adult beetles to prevent oviposition in horticultural crops is not yet sufficiently solved. Only chemical insecticides or labour intensive collection are possible. According to the efficacy of EPN as a potential agent in controlling the imagines of *Curculio caryae* or *Blattodea* spp., a laboratory trial with *Steinernema carpocapsae* against beetles of *O. sulcatus* was carried out. Boxes ($20 \times 20 \times 5$ cm) were filled up to 0.5 cm with a mixture of sand and peat based substrate (v:v = 1:1). A small shoot of *Taxus baccata* and 4 cm^2 of wet filter paper were added. Each of the 8 cages (treatment and control in 4 replicates) included 5 beetles. A trap was developed for EPN application (patent pending) consisting of a gel-like formulation filled into 3 grooves (1 ml each) under a 90 cm^2 artificial, wooden shelter. Untreated controls consited of the trap with the gel-like formulation without nematodes. The artificial shelters were placed into the boxes one day after the beetles. The trial was located in a greenhouse cabinet with natural daylight (approx. 12:45 hours) and an average air temperature of 19.5 °C (SD = 3.9 °C). After 14 days the number of living and parasitized *O. sulcatus* was determined. All of the 20 beetles in the *S. carpocapsae* treatment were killed, in contrast to the control, where all the beetles were still alive. The result of 100% mortality clearly indicates that not only larval and pupal stages of *O. sulcatus* are susceptible to EPN, but also adults can be parasitized. The successful control of the beetles might relate with the need of the weevils for a shelter during daylight, where they were exposed to nematodes for a longer time to facilitate nematode infestation. The innovative formulation can prolong nematode survival and produce favourable conditions for infestation. Further trials in larger scale are necessary to assess the suitability of this method for horticultural purposes. A combination with an attractant resulting in an "attract and kill" strategy for *O. sulcatus* adults would be of special interest.

Influence of climate change on insects and natural pest in horticulture

C. Tölle-Nolting, R. Meyhöfer & H.-M. Poehling

Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Hannover

The climate will continue to change within the next years as it did in the last century. Important changes are related to the amount of greenhouse gases in the atmosphere and current enrichments are followed by increasing temperatures (warmer winters and nights) and more extreme changes in short term periods of heat and precipitations (heavy rains and dry spells). Besides several direct and indirect large scale impacts on ecosystems it is likely that horticultural pest and their natural enemies are affected.

The most important factors for insect development are temperature and precipitation. It has been supposed that under a warmer climate, species will move to more northern areas and that species, which cannot migrate, will diminish their area but increase in their abundance. At higher temperatures insects develop faster and more generations of pest are expected. The warmer winters will decrease winter mortality and the pests will reach the plants in a more vulnerable stadium. But very hot temperatures will increase the mortality of insects. A change in the temperature can also lead to an asynchrony between prey and predator/parasitoid, if they are triggered by different abiotic factors, e.g. photoperiod and temperature. The development of the dark-coloured caterpillar *Melitaea cinxia*, for example, is depending on the sunlight, while its light-coloured specialist parasitoid *Cotesia melitaearum* is mainly influenced by air temperature (Van Nouhuys and Lei 2004). Precipitation is another important factor for insects. Rain can prevent the development of insects and heavy rains can wash the insects off the leaves and increase mortality. In return drought can make the plants more vulnerable for herbivores due to a decreased nutrient uptake and a decreased level of secondary compounds. But the different feeding guilds react in a different way to drought. Gall formers are likely to react negatively, chewing insects are not reacting to moderate drought and sucking insects seem to react positively. Parasitoids seem to deal better with dryness, but all respond negatively to heavy droughts.

Hence we need more information about variation in pest/natural enemy interaction pattern under different temperature/humidity scenarios and under realistic experimental conditions before reliable predictions can be performed. It is the aim of the KLIFF-Network (KLImaFolgenForschung in Niedersachsen) to study the influence of climate change on agriculture, forestry and water management and develop adaptation strategies. In particular we will focus in our sub-project on the reaction of some of the coming pest species in horticultural crops and interactions with natural enemies under changing temperatures and precipitation.

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The determination of temperature as an important driving force for population dynamics processes of coccinellids as aphid predators

S. Krengel & B. Freier

Julius Kühn-Institut, Institut für Strategien und Folgenabschätzung im Pflanzenschutz, Kleinmachnow

The ladybirds *Coccinella septempunctata* and *Propylea quatuordecimpunctata* belong to the most important natural enemies of cereal aphids. Since a couple of years the invasive species *Harmonia axyridis* increasingly colonizes maize and other arable crops such as wheat fields. There is indication that the expected global warming will change the predatory potential and the intraguild relationships of these species. Possible differences between the species *Coccinella septempunctata* and *Harmonia axyridis* are matter of particular interest. Aim of the present study was the systematic analysis of currently existing knowledge on the temperature as driving variable in population dynamics of coccinellids and identification of existing knowledge gaps concerning the influence of elevated temperatures on coccinellids and their regulatory effects.

The compartment network approach was used. That means, the population of each coccinellid was divided into different compartments and the most important state variables, such as immigration, development, reproduction, feeding and survival, were determined for each compartment. Because the effect of temperature as the most important driving variable for these state variables varies, the determination of these relationships was necessary for each state variable separately.

The collected data from previous investigations and literature showed indications of a relative similarity of the temperature-dependent reactions of the two species *Coccinella septempunctata* and *Harmonia axyridis* in some important processes. The two species are nearly comparable in development thresholds, temperature optima and duration of development. However, they differ considerably, for example, in oviposition rate. In 2008/09, some laboratory experiments at the Julius Kuehn-Institute concerning mortality in regard to different temperature regimes were performed. These investigations indicated differences between the two species *Coccinella septempunctata* and *Harmonia axyridis*.

However, our knowledge about that influence is still very incomplete. In particular, there is a deficiency of comparative investigations. In addition, divergent investigation conditions, such as temperature regime used, nutrition quality and quantity as well as duration of investigation, complicate the secondary analysis of existing data. Within a new climate chamber, used for the so-called “Arche Noah Experiment”, conditions can be better controlled. Preliminary results of this experiment with defined predator communities showed advantages for *Harmonia axyridis* over *Coccinella septempunctata* at higher temperature as a result of different effects, such as intraguild competition and predation.

Analysis of virulence of Baculoviruses to improve the biological control of cutworms (*Agrotis* sp.)

J.T. Wennmann & J.A. Jehle

Julius Kühn-Institute, Institute for Biological Control, Darmstadt

Different cutworm species are serious economic pests of most vegetable and field crops nearly all over the world. They are extremely difficult to control due to their soil dwelling behaviour. In Germany, the economically most important cutworm is *Agrotis segetum*, whereas in Egypt, the black cutwom *A. ipsilon* is a severe pest. In recent years, the black cutworm became an increasingly important pest in Middle Europe. An ecologically sound control method for many agricultural pests is the application of baculoviruses, which are insect-pathogenic viruses and have been developed to highly specific and environmentally benign insect control agents. Cutworms can be infected by three different baculoviruses AgseNPV, AgipNPV and AgseGV. We aim to study the virulence mechanisms of these viruses by molecular tools and hence improve the application of these viruses as biological control agents. By applying bacmid technology, candidate virulence genes of the

viruses will be identified and functionally characterized. Knowledge of virulence genes is a prerequisite to optimize the application of these viruses. The efficacy of the viruses will be tested in greenhouse and/or field experiments. In co-operation with a biopesticide producing company a product will be developed.

This project shall result in the optimization of the control of different cutworm species with different baculovirus isolates. By identifying and characterizing genes involved in the virulence, basic knowledge on the genetic requirements for their specific infectivity towards different cutworm species will be obtained. In collaboration with a company, experienced in producing and formulating baculovirus bio-control agents, test formulations of these viruses will be produced and tested in greenhouse and field experiments in Egypt and in Germany. The project will help to develop environmentally safe biological control methods of a pest species complex, which is extremely difficult to control and that will reduce the application of more toxic chemical insecticides and hence will also reduce exposure of consumers to chemical residues.

The South American Tomato Moth, *Tuta absoluta*, a new pest in Germany: An assessment of biological control options

O. Zimmermann & B. Wührer

AMW Nützlinge GmbH, Pfungstadt

The South American tomato moth, *Tuta absoluta* (Gelechiidae), has been recorded in Europe since 2006. It was introduced from South America and has spread all over Europe. It is already among the most important tomato pests in Spain. High economic losses are expected, e.g. in the Netherlands additional insecticide treatments against *T. absoluta* will cost up to 4 million Euro per year. In 2009, *T. absoluta* was observed in Germany for the first time.

The larvae are mining in leaves and stems of their host plants and can reach up to nine generations per season in greenhouses. The adult moth is unremarkably coloured and spreads actively to other locations. Host plants are Solanaceae and beside tomatoes include potatoes and ornamental *Solanum* species. An effective control of *T. absoluta* is difficult.

Chemical insecticides are not sustainable because they can lead to resistance and residue problems on tomato fruits. Pheromone traps are being offered for monitoring and mass trapping but can only be part of a control strategy. Natural enemies and insect pathogens (viruses, *Bacillus thuringiensis*, entomopathogenic fungi, nematodes, larval parasitoids) are principally suitable, but there is little or no practical experience with them.

In Spain, egg parasitoids (*Trichogramma achaeae*) are combined with predatory bugs (*Nesidiocoris* sp., *Macrolophus* sp.). But they are slow in controlling the pest and have to be used at high dosages. Such a control strategy could also be adapted for Germany but must be optimized and modified to using indigenous beneficial species. There are scientific results that show that *T. achaeae* which is being used in Spain cannot overwinter in Central Europe. This contradicts reports of *T. achaeae* being a cosmopolitan species. First experiments on suitable species for Germany are in progress. The Potato Tuber Moth *Phthorimaea operculella*, which is closely related to *T. absoluta*, has been controlled successfully in a greenhouse by using the TrichoKarte „Gewächshaus“, which employs a mixture of indigenous *Trichogramma* species.

A suitable integrated control strategy for *T. absoluta* in Germany is urgently needed because of the anticipated high economic losses and the risk of abandoning the long-term successes of biological control in tomato greenhouses and reverting to insecticide treatments and its disadvantages. The biological control of *T. absoluta* is possible in principle, but needs further investigations.

Preliminary trials to evaluate efficacy of stable fly parasitoids for control of tephritid fruit fly pests

A. Herz[1], N. Becker[2], K. Köppler[3] & H. Vogt[3]

[1] Julius Kühn-Institute, Institute for Biological Control, Darmstadt; annette.herz@jki.bund.de
[2] University of Applied Sciences, Geisenheim
[3] Julius Kühn-Institute, Institute for Plant Protection in Fruit Crops and Viticulture, Dossenheim

Several species of the Dipteran family Tephritidae belong to the most serious fruit pests worldwide. Polyphagous species like the Medfly, *Ceratitis capitata* Wiedemann, but also more specialized ones (eg. species of the genus *Rhagoletis*) are difficult to control due to the lack of registered efficient pesticides. Releases of effective parasitoids may help to improve biological control of these pests. Literature reports on the use of Pteromalidae (Hymenoptera, Chalcidoidea), practically used for stable fly control, against *C. capitata* in Spain and South Africa, caused us to test several commercially available parasitoid species for their capacity to parasitize puparia of *C. capitata*, *Rhagoletis cerasi* L., the European cherry fruit fly, as well as *R. completa*, a North-American species developing on walnut and currently invasive in Europe. At first, mated females of the Pteromalids *Nasonia vitripennis* (Walker), *Spalangia cameroni* Perkins, *Muscidifurax raptor* Girault & Sanders and *M. zaraptor* Kogan & Legner were subjected in a non-choice situation to puparia of the particular Tephritid species or puparia of the blow fly *Lucilia sericata* Meigen, a preferred host of all tested parasitoid species. *N. vitripennis* readily accepted *L. sericata* as host (95% of offered puparia parasitized) but refused *C. capitata* as well as *R. cerasi* in this experiment (0% parasitism). In contrast, *M. raptor*, *M. zaraptor* and *S. cameroni* were able to parasitize the Tephritid puparia, although *S. cameroni* attacked puparia of *R. cerasi* (8% parasitism) to a much lesser extent than those of *C. ceratitis* (54% parasitism). *M. raptor* and *M. zaraptor* also accepted puparia of *R. completa*. In a next step, the preference for a particular host species was tested in a choice situation (puparia of Tephritidae versus those of *Lucilia*, *Rhagoletis* versus *Ceratitis*). *M. zaraptor* parasitized puparia of *C. capitata* more successfully than those of *L. sericata* (0.9 eggs/female versus 0.67 eggs/female within 4 h of contact) or *R. cerasi* (0.7 eggs/female versus 0.4 eggs/female within 4 h of contact). The other parasitoid species preferred the larger host, *L. sericata*. Further trials will evaluate the searching efficacy of the different parasitoids (*S. cameroni*, *M. raptor*, *M. zaraptor*) for location of puparia buried in the soil as it is the case in the field situation.

Preliminary results of trials to control the corn rootworm with entomopathogenic nematodes

P. Knuth

LTZ Augustenberg, Außenstelle Stuttgart, Stuttgart

Since abandoning the seed treatments with neonicotinoide insecticide the chemical control of the Western corn rootworm (*Diabrotica virgifera virgifera*) in Germany became rather difficult and subject of controversial public discussions. Results provided by CABI Europe from Hungary in 2005/2006, indicate that the application of entomopathogenic nematodes, in particular *Heterorhabditis bacteriophora*, is considered as an effective tool to control larvae of the Western corn rootworm. The LTZ has launched a research project in 2009 aiming to explore the survival of the antagonist under climate and soil conditions of the Upper Rhine Valley, when the nematode is released during sawing. In an infested field, rootworm larvae emerge about six weeks after sowing. Accordingly, the survival of the nematode for several weeks without host larvae is

a vital prerequisite. In addition, it is necessary to elaborate a practical technology for nematode application, in particular application technology, date and type of treatment (liquid or granules), application rate of the nematode and to assess efficacy and profitability.

The trails were conducted in the vicinity of Freiburg on two fields with sandy and loamy soils, respectively. Seven treatments were set up at each of the two sites. These included: (1) nematodes applied in suspension of 200 l/ha applied at sowing into the seed furrow at a rate of 112,000 larvae per row meter or 1.5 billion per ha, (2) nematode suspension as treatment Nr.1 but with 400 l/ha water volume, (3) nematode suspension as treatment Nr.1 placed 5 cm aside the seed furrow in 5 cm below the seed level, (4) same treatment as Nr. 3, but with 400 l/ha water volume, (5) applied as granules at sowing into sowing row at the rate 10 kg/ha (corresponding to 150,000 larvae per g), (6) nematode suspension with 200 l/ha as a post emergence application during the 4-leaf stage of corn, about 15 cm aside the corn row and in 15 cm depth, (7) nematode suspension as treatment Nr. 6, but with 400 l/ha water volume. Nematode survival in the soil was assessed by a standard bioassay. Twenty individual soil samples/treatment were taken in weekly intervals (per treatment) within the interrow space. Each of these samples received 20 mealworms and was incubated at room temperature. The rate of mealworm parasitism by the residual nematodes was then recorded. Surprisingly, even 10 weeks after application at low rates *H. bacteriophora* was *bacteriophora* still parasitizing mealworms in the bioassay. The soil type had a significant impact on the mortality of the mealworms. Mortality of the mealworms in loamy soils (treatments Nr 1 and 2) reached 45% even four weeks after application, whereas in the sandy soil mortality dropped below 30% after four weeks. Due to the poor flow ability the results obtained with the granules are not realiable. Presumably, only 3 kg/ha instead of 10 kg/ha were applied and this might not have been sufficient for the required control rate. Unexpectedly, the application of the nematodes as granules produced a mortality of mealworms of about 30% after 4 weeks. Assuming the granules formulation can be improved in terms of flowability, this application method will certainly be a very promising option, since the necessary equipment is likely to be available for most farmers. These preliminary results obtained in the first year are quite promising for the biological control of the corn rootworm in the State of Baden-Württemberg.

Initial investigations on the ability of the indigenous larval parasitoid *Bracon brevicornis* to control the Box Tree Pyralid *Diaphania perspectalis* in Germany

O. Zimmermann & B. Wührer

AMW Nützlinge GmbH, Pfungstadt

Since its first appearance in the Upper Rhine Valley in 2007, the neozoic Box Tree Pyralid *Diaphania perspectalis* (= *Glyphodes perspectalis*) has locally established on *Buxus* sp. in home gardens and parks in Germany. It was found mainly along the river Rhine and in nearby regions. The larvae overwinter as L2 larval stages and can already start causing new damages in March. *D. perspectalis* is actively migrating into new areas and its spread might be enhanced by repeated introductions of infested plants. Thus, it is highly recommended to carefully check imported *Buxus* sp. plants. For controlling the Box Tree Pyralid, advisory services currently recommend the biological products Neem and *Bacillus thuringiensis*, as well as chemical pesticides (e.g. thiacloprid). Spraying insecticides has only limited success because the pyralid larvae are protected by their webbing. The potential of using parasitoids has, so far, received little attention although it could be a biological and residue-free alternative for gardens and parks.

Except for laboratory trials with the egg parasitoid *Trichogramma brassicae* there is no information on parasitoids of *D. perspectalis* in Germany. Therefore, the suitability of this potential antagonist against the Box Tree Pyralid was investigated in laboratory experiments. The objective of an integrated control strategy could be to reduce the development of the pest populations during spring by controlling the overwintering larvae through the release of larval parasitoids and the ovipositing adults with *Trichogramma* wasps. As part of a research project on the control of the European Corn Borer *Ostrinia nubilalis* the indigenous braconid larval parasitoid *Bracon brevicornis* is available through a large scale rearing.

Larvae of *D. perspectalis* (last larval stage) were exposed to single parasitoid females of *B. brevicornis* in Petri dishes. The parasitization was observed for one week. After four days, 87% of the larvae were paralysed, which means an irreversible immediate stop of feeding activities. Oviposition was observed on 77% of the paralysed larvae. However, the larvae of the braconid wasp could not develop in *D. perspectalis* and died. Possibly, alkaloid substances of the Box Trees negatively influenced the development of the parasitoids. In any case, the host-feeding did not result in immediate adverse effects on the parasitoid adults. On average, 14 eggs were laid on each pyralid larva during a period of four days. Frequent host-feeding activities of the braconid wasps were observed. They sting or bite the host larva, perforating the host body, and feed on the haemolymph. The trials have shown that *B. brevicornis* accepts *D. perspectalis* as a host, but cannot complete its development in it. In 2010, the potential of the combined use of braconid wasps and *Trichogramma* species will be tested under field conditions.

Behaviour and development of the parasitoid *Bracon brevicornis* – an enemy of the European corn borer *Ostrinia nubilalis*

U. Wyss[1], B. Wührer[2] & O.Zimmermann[2,3]

[1] Institut für Phytopathologie, Universität Kiel
[2] AMW Nützlinge GmbH, Pfungstadt
[3] JKI Darmstadt

In recent years, in Germany, the ectoparasitic wasp *Bracon brevicornis* (Hym. Braconidae) was quite often detected parasitising *Ostrinia nubilalis* larvae in infested maize crops. For this reason a video film (duration 14 $^1/_2$ min.) was produced that documents the behaviour and development of the braconid parasitoid. Infested maize stalks were partially cut open to reveal the host larvae (L4 – L5) and were then presented under a stereomicroscope to mated *B. brevicornis* females. As a typical response, the *Ostrinia* larvae first tried to protect themselves by spinning a web within the cut stalk that was then partially covered by frass and faeces. The wasp, standing nearby, continuously observed this mending process with great care, waiting for the right moment for the first sting to paralyse the mighty larva by injecting a venom. Several attempts with the partially protruded ovipositor preceded the first very quick successful sting, upon which the host larva responded violently. Several minutes elapsed, continuously observed by the parasitoid, until the affected larva gradually calmed down. Then it was stung again.The paralysed *Ostrinia* larva was then removed from the stalk to document host feeding and oviposition. First host feeding stings evoked again a strong defensive response. Finally, after the larva had calmed down completely, host feeding was initiated by forceful deep ovipositor stings. The emerging haemolymph was immediately imbibed. Host feeding was followed by protruding the ovipositor at almost full length along the body of the host. The egg was then seen to flow out from a lateral slit of the ovipositor. Oviposition of two successive eggs could be documented. Subsequent sequences show that many (up to 30) eggs are

deposited at different portions of the paralysed, still pulsating host. The development of the parasitoid from egg deposition until adult emergence was recorded at 27–28°C. Emphasis was placed on the following features: Embryonic development, hatch of the L1 larvae that immediately start feeding, food ingestion at high magnification, the rapid growth of the larvae until they are fully developed after about three days. These larvae start to spin a dense silken pupation cocoonl. Final sequences show the praepupa and pupa within the cocoon and how, five days after the onset of pupation, an adult male and female emerged from the cocoon.

Effects of imidacloprid and thiamethoxam applied on sugar beet seeds on *Poecilus cupreus* larvae

B. Baier & D. Schenke

Julius Kühn-Institute, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin

Imidacloprid and thiamethoxam, neonicotinoids with different chemical properties, were investigated to determine their effects on *Poecilus cupreus* when applied on sugar beet seeds. For this purpose, laboratory test were carried out with larvae of this carabid beetle in natural substrate (Lufa 2.1). The purchased sugar beet seeds that had been coated with either Gaucho® WS 70 (imidacloprid) or Cruiser® 70 WS (thiamethoxam) were characterised by the corn weight and the content of active ingredient per seed. Used seeds contained 781 (± 147) µg imidacloprid/seed and 749 (± 96) µg thiamethoxam/seed, respectively. The tests were carried out in plastic boxes with different surface areas (384, 188 and 92 cm^2) but the same height (6 cm). Additionally, glass tubes (2,5 cm diameter, 7 cm height and 5 cm^2 inner surface) were used. This setup simulated a 2, 4, 8 and 154-fold seed density in relation to the maximum field rate of 130,000 sugar beet seeds/ha. Each test unit was equipped with one coated seed in the middle of the unit 1.5 cm deep within the soil and one 24 to 48 h old larva. Apart from investigation of the efficacy towards the carabid beetle, the diffusion of the active substances imidacloprid and thiamethoxam from the seeds into the soil was determined by residue analyses of soil samples in different distances from the seed. The lethal effects of imidacloprid on *Poecilus cupreus* decreased with increasing surface area of the boxes. The lethal effect was 100% in the case of the 154-fold seed density and only 6% in the case of the twofold seed density. In comparison, thiamethoxam caused > 90% mortality in all simulated cases of seed density. During the whole test period, the residues of imidacloprid in the closer soil fraction remained closely around the seed (r = 1.2 cm). Thiamethoxam was distributed in a significantly larger content in a radius of 3.6 cm compared to imidacloprid. The greater water solubility of thiamethoxam was presumably the reason for a clearly greater seed dressing zone of this substance. Therefore the higher probability of contact between *Poecilus cupreus* and thiamethoxam could be one reason for the more pronounced effects in comparision to imidacloprid.

Control of the woolly apple aphid (*Eriosoma lanigerum* Hausm.) by releasing earwigs (*Forficula auricularia* L.) and support oil applications

I. Toups[1], J. Zimmer[1], M. Trautmann[2], M. Beer[3], S. Buchleither[4] & A. Herz[5]

1 DLR Rheinpfalz, Kompetenzzentrum Gartenbau, Rheinbach
2 Kompetenzzentrum Obstbau Bodensee, Ravensburg
3 Öko-Obstbau Norddeutschland Versuchs- und Beratungsring e.V., Jork
4 Beratungsdienst Ökologischer Obstbau e.V., Ravensburg
5 Julius-Kühn Institut Darmstadt, Institut für biologischen Pflanzenschutz, Darmstadt

In order to develop an on-farm strategy to control the woolly apple (*Eriosoma lanigerum* Hausm.) aphid in organic orchards a research project funded by the Federal Agency for Agriculture and Food, Germany, was conducted from 2007 to 2009 in cooperation with different research facilities in Germany. The focus was directed at the release of the common earwig (*Forficula auricularia* L.), possibilities to enhance populations in the field and applications of oils to control the woolly apple aphid. Additional trials were conducted to determine the pollution of apples by earwigs' excrements and the influence of mechanical soil management on the overwintering earwigs. Round robin tests in the field where made in cooperation with fruit growers in the region of Lake Constance. The healthy stages of the earwigs from all sites were examined in laboratory. Earwigs as natural predators of woolly apple aphids climb the trees at the end of May/beginning of June. By then the population of woolly apple aphid may have reached high infestation levels. To keep infestations at moderate levels until the earwigs appear, oil applications were made in early spring to complement the release of earwigs. Results show that the efficacy of released earwigs was hardly determinable and depended on the prior infestation intensities. When the earwigs appear in the trees lots of other beneficial organisms were found, like ladybeetles (Coccinellidae) and their larvae as well as larvae of hover flies (Syrphidae) and green lacewings (Chrysopidae), which all feed on woolly apple aphid and seem to obscure the earwigs` predation. The trials included comparisons between oil application by brush and by spraying in combination with earwig release, respectively. The brushing was made in April when the first woolly colonies can be found in the orchards. For better efficacies the spraying must be accomplished before the aphids starts to produce their woolly cover. At high infestation levels the oil application by brushing proved to be much more effective, but is time consuming. The efficacies for spraying were highly variable. At low infestation levels an oil application in April before the aphids start to produce their woolly covers seems to limit the development of the aphids. However at higher infestation levels a spraying seems to have no influence on the population development. The release of earwigs alone was not sufficient to control a high infestation of woolly apple aphid. A combination of long term promotion of earwigs' population with an oil application (brushing or spraying) in early spring, together with the impact of all natural enemies including *Aphelinus mali* can be a promising strategy to keep the infestation by woolly apple aphid at reasonable levels.

Earwigs can become sick too!

U. Koch, H. Bathon, A. Herz & R.G. Kleespies

Julius Kuehn-Institute, Institute for Biological Control, Darmstadt, Germany; annette.herz@jki.bund.de

Common earwigs (*Forficula auricularia* L.) are important predators of serious pests in fruit growing, e.g. the woolly apple aphid (*Eriosoma lanigerum* Hausm.). Enhancement of natural pest control was tested in a joint research project by collecting of earwigs from orchards with high population densities and subsequent release into plantations heavily infested by the woolly apple aphid at several locations in Germany (see Toups et al., 2010, within this issue). Releasing beneficials, which had been collected in nature for biocontrol, may pose the risk to transfer parasitoids and diseases from one population to another. To evaluate this risk, samples of several hundred earwigs were taken from the different collections and analyzed for their health status in the laboratory. After freez-

ing, earwigs were dissected and examined for the presence of macroorganisms (larvae of parasitoids, nematodes etc.) under the stereomicroscope. Smear tissue preparations with subsequent Giemsa staining were made from selected individuals of the samples and investigated by light microscopy. In some cases examinations by electron microscopy were conducted. Less than 10% of the different earwig populations from Lake Constance region, Rhineland-Palatinate and Lower Saxony were found to be parasitized during the three years of the study. *Triarthria setipennis* Fallén (Dipt., Tachinidae) was the dominant parasitoid in samples from Lake Constance region (2007: 49 parasitized out of 1187 dissected, 2008: 27 out of 663, 2009: 28 out of 987). Larvae of this tachinid were also found in earwigs from other locations. The nematode *Mermis nigrescens* Dujardin (Nemtoda, Mermithidae) was detected in three earwigs from samples taken in 2007 at the Lake Constance region and in higher numbers from earwigs collected in 2008 and 2009 at all locations (Lake Constance region: 13 of 663 dissected in 2008, 84 of 987 in 2009; Rhineland-Palatinate: 36 of 689 in 2008, 55 of 783 in 2009; Lower Saxony: 26 of 823 in 2008, 7 of 740 in 2009). Pathogenic microorganisms were also detected. Microsporidia were isolated for the first time in earwigs from all locations. Infection studies have to be conducted to clarify life cycle and ultrastructure of all developmental stages. Light and electron microscopy as well as phylogenetic analyses will be necessary for final determination of these cell parasites as well as of other pathogens, found in a few specimens from the Lake Constance region. According to these results, the health status of natural earwig populations can be considered as satisfactory and it seems that the most important antagonists of earwigs already occur in different regions in Germany thus limiting the risk of contamination of earwig populations by active release of specimen from other regions.

A cDNA-AFLP and qRT-PCR approach to identify genes involved in CpGV-resistance of the codling moth, *Cydia pomonella*

N.A. Gund & A. Reineke

Geisenheim Research Center, Department of Phytomedicine, and University of Hohenheim, Institute of Phytomedicine, Department of Applied Entomology, Stuttgart

The codling moth, *Cydia pomonella*, occurs worldwide in apple growing regions and is one of the most serious insect pests in apple orchards. The larvae are polyphagous and can also damage quinces, cherries, plums, apricots, walnuts, and pears. Larvae tunnel into the fruits to the core where they feed on developing seeds. Without any control, damage can reach up to 95% in an apple orchard. The *C. pomonella* granulovirus (CpGV), Family Baculoviridae, is one of the most powerful tools for reducing *C. pomonella* populations, especially in organic farming. Since 2003, less sensitive codling moth populations against this virus emerged in Germany and in other European countries (France, Italy, Switzerland, the Netherlands and Austria). The populations had a up to 1000-fold reduced sensitivity against the Mexican isolate of this virus, CpGV-M. Single-pair cross experiments indicted that the putative dominant CpGV-M resistance gene is located on the Z-chromosome (sex chromosome) of the codling moth (Asser-Kaiser et al. 2007).For identifying genes prospectively involved in the development of CpGV resistance, a gene expression profiling approach was chosen. CpGV-M resistant codling moth larvae in the fourth instar were exposed for a certain period of time to virus-contaminated and virus-free diet via "droplet feeding". Two different CpGV strains were involved in this assay: CpGV-M, for which resistance is know to exist in the field, and CpGV-I12, a new isolate from Iran, with apparent resistance-breaking effects. As a control a virus-free diet was used. Complementary DNA-amplified fragment length polymorphism (cDNA-AFLP) analysis and subsequent quantitative Real Time-PCR (qRT-PCR) were applied to identify and to compare the expression levels of different genes putatively involved in the resistance process between the different samples. Genes, which were differentially expressed in *C. pomonella* larvae fed on virus-contaminated or virus-free diet where isolated from cDNA-AFLP gels and were sequenced. Among them, one candidate gene showed a high homology to insect intestinal mucins, which are known to be involved in defence reactions of insects against pathogen infections. Expression of this gene after various time points of CpGV infection will be monitored in the future.

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Influence of humidity, water application volume and a formulation on the control potential of the entomopathogenic nematode *Steinernema feltiae* on overwintering larvae of the codling moth *Cydia pomonella*

T. Navaneethan & R.-U. Ehlers

Institute for Phytopathology, Dept. of Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Kiel; ehlers@biotec.uni-kiel.de

Codling moth (*Cydia pomonella* L.) is a serious pest of pome fruit. Diapausing cocooned larvae overwinter in cryptic habitats in the soil around or the bark of infested trees. The entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) is used to control diapausing codling moth larvae. However, efficacy can be variable. The objective of this study was to define environmental conditions favouring the performance of the nematodes. Virulence of *Steinernema feltiae* was superior to *S. carpocapsae*. Cocooned larvae were more susceptible than non-cocooned larvae. Mortality of pupa was low. The humidity in the substrate was assessed measuring the water activity (a_w-value). *S. feltiae* was unable to infect larvae at a_w-values ≤ 0.9. Cocooned larvae died at lower a_w-values than non-cocooned larvae. Mortality of cocooned larvae did not further increase after half an hour of exposure, whereas the mortality increased with increasing exposure time in non-cocooned larvae. LC_{50} and LC_{90} considerably decrease with increasing relative humidity in the air. The negative influence of the relative humidity was less important at a relative humidity surpassed 80% than the effect of water activity in the substrate, which can be increased by spraying larger water volumes. When *S. feltiae* was formulated in a surfactant-polymer-formulation, mortality significantly increased when compared to application in water only. In summary, following recommendations can be drawn from the results: 1. Application should be against cocooned larvae, because they are more susceptible. 2. Relative humidity should at least be at 80% during application and few hours after application. 3. The lower the relative humidity, the high should be the application volume of water. 4. The surfactant-polymer-formulation should be used, particularly when suboptimal environmental conditions cannot be expected.

Heat and desiccation tolerance of selected hybrid strains of the entomopathogenic nematode *Heterorhabditis bacteriophora*

J. Mukuka & R.-U. Ehlers

Institute for Phytopathology, Dept. of Biotechnology and Biological Control, Christian-Albrechts-University Kiel, Kiel; ehlers@biotec.uni-kiel.de

Genetic selection can be a powerful tool to increase beneficial traits in biological control agents. Heat and desiccation tolerance of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (Rhabditidomorpha: Strongyloidea) were significantly increased by cross breeding tolerant parental strains and successive genetic selection. These strains originated from a prior screening among 60 strains for increased stress tolerance. During genetic selection, the selection pressure was constantly increased and only the most tolerant 10% of the nematode populations were propagated for further selection steps. Assessment of tolerance and selection for both traits was performed with and without prior adaptation to the stress conditions. Eleven selection steps were performed to increase heat tolerance. A final overall increase in mean heat tolerance of 5.5°C was achieved when nematodes had been adapted to heat stress. For non-adapted tolerance an increase of 3.0°C from 40.1° to 43.1°C was recorded. For comparison, a commercial strain had a mean tolerated temperature after adaptation of 38.2°C and of 36.5°C without adaptation. For assessment of the desiccation tolerance the mean tolerated water activity (a_w-value) of a population was measured. Cross-breeding most tolerant strains reduced the a_w-value from 0.67 to 0.65 after adaptation and from 0.9 to 0.7 without prior adaptation. The following six selection steps could not increase the tolerance whether nematodes had been adapted to stress or not. In comparison, the commercial strain tolerated a mean a_w-value of 0.985 after adaptation and 0.951 without adaptation. Further investigation will have to assess trait stability and possible trade-off effects. This study is a first important step on the road towards domestication of the entomopathogenic nematode *H. bacteriophora*.

Complementary sex determination and inbreeding avoidance in the parasitic wasp *Bracon brevicornis*

A.C. Weeda, A. Thiel & T.S. Hoffmeister

Population Ecology Lab, University of Bremen, FB2, Bremen; weeda@uni-bremen.de

In species with single-locus complementary sex determination (sl-CSD), sex is determined by multiple alleles at a single locus. In the haplodiploid Hymenoptera, sl-CSD results in females, if individuals are heterozygous (2n) at the sex locus, and in males, if they are hemi- (n) or homozygous (2n). Diploid males originate from matched matings, i.e. if a female wasp mates with a male carrying a sex allele matching one of hers. Given successful development, they are sterile and, additionally to having zero fitness, accrue costs on females they mate with. As a consequence of matched matings, parasitic wasps with sl-CSD rapidly show effects of inbreeding depression in small populations. In nature, females of *Bracon brevicornis* produce clusters of eggs when parasitizing host insects and thus, there is a large potential for inbreeding through sib-matings. This is especially for mass rearing in biological control programmes. An obvious question is how *B. brevicornis* may avoid the associated costs. We investigated three different potential mechanisms of inbreeding avoidance. 1) Males and females may emerge asynchronously and may not meet on the natal patch due to immediate dispersal. 2) Females may reject mating attempts early in their life and thus, before dispersal and 3) females may employ kin-recognition to reject brothers as mating partners. Our experiments suggest that by appropriate mating systems, this parasitoid has behavioural mechanisms that allow to reduce the severe costs thought to be associated with sl-CSD.

Banker plant system for predatory flies of the genus *Coenosia* Meigen, 1826

S. Kühne[1], D. Pohl[2] & J. Klatt[3]

[1] Julius Kühn-Institute, Kleinmachnow; stefan.kuehne@jki.bund.de
[2] Süleyman Demirel University, Plant Protection Department, Isparta, Turkey
[3] Landwirtschaftskammer Nordrhein-Westfalen, Münster

Predatory flies of the genus *Coenosia* (Diptera: Muscidae) are polyphagous predators and feed on important greenhouse pests, including whiteflies (Aleurodidae), fungus gnats (Sciaridae) and leafminers (Agromyzidae), but also on leafhoppers of the genus *Eupteryx* and *Empoasca* and small dipteran species (Ephydridae, Drosophilidae) (Kühne 1998). The wide range of prey used as food makes them very flexible, hence especially useful (Kühne 2000). Furthermore, they are the only beneficials that can kill adult stages of these pests. In 1996–2000 *Coenosia attenuata* Stein, 1902 were first applied against the mentioned pests in ornamental and vegetable (cucumbers, tomatoes) crops on more than 20 ha of greenhouse area in Germany. In some big enterprises like Anthura Arndt GmbH (Borken-Burlo) *Coenosia* flies have been established since 1999 and help to reduce the whole costs for biological control. Their presence is also an indicator for reduced pesticide application. In 1998, *C. attenuata* was first found in cotton fields in Turkey (Pohl, 2003). In 2002, *C. attenuata* was recorded for the first time in greenhouses from the Neotropical Region in Ecuador and Peru (Martinez-Sanches et al. 2002). The efficacy of *C. attenuata* as a biological control agent of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) in greenhouse vegetables was studied in Portugal and Spain (Aguilera 2004, Rodriguez et al. 2004, Pinho et al. 2009, Tapia et al. 2008). In 2009, the authors discovered *C. attenuata* in greenhouses in Antalya, Turkey, for the first time and a greenhouse experiment was set up to establish *C. attenuata* by offering one box with plantation substrate (coconut fibre) for egg depositing and as a food source. The substrate was mixed with oat flakes. Fungus mycelium was growing on the oat flakes in the substrate and provided the basic food for fungus gnats larvae. Because *Coenosia* larvae feed on fungus gnat larvae and the adult *Coenosia* feed on adult fungus gnats we succeeded to establish the predators within the greenhouse for more than three months. Further investigations were interrupted due to other use of the greenhouse. The establishment of *Coenosia* by release of 1100 flies in a greenhouse of the Botanical Garden in Berlin in 2009 was not successful. We assumed that geckos living in the greenhouse fed on the flies and cockroaches nesting in the rearing substrate destroyed the food source for *Coenosia*.