

Report on the 36th Annual Meeting of the Working Group “Beneficial Arthropods and Entomopathogenic Nematodes”



Our 36th meeting of the working group was held at the Botanika in Bremen, situated in the Rhododendron Park holding the world's second largest rhododendron collection with more than 600 species and numerous breeds of rhododendron. So, our meeting was delightfully opened by the director, Dr. H. Schepker, who took us on a virtual tour through the history, role and beauty of the Rhododendron Park and the exhibition halls of Botanika, but also explained us daily practice of park management including plant health problems. Then 17 talks followed including a poster session. Highlight of the late afternoon was the presentation of three new fascinating videos by Prof. Urs Wyss (University of Kiel, entofilm) showing in close-up view the battle between parasitoids or predators and their prey (see abstracts below).

We would like to thank all participants, especially those which provided us with short abstracts of their contributions compiled in this report.

The next meeting will again be organized together with the “Arbeitstagung Biologische Schädlingsbekämpfung”. We have been invited by Mrs. Dr. M. Zunker to meet at the LTZ Augustenberg near Karlsruhe from November 26th to 27th 2019. Please expect our invitation in late summer 2019.

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

An attract and kill formulation for fungus gnat control combining an entomopathogenic fungal strain and a botanical compound

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Fungus gnats (*Bradysia* spp.; Diptera: Sciaridae) are major insect pests of greenhouses and nurseries. Organic production systems need to fall back on biological control strategies for fungus gnat control. Several microbial products and biorationale insecticides have been developed and evaluated as pest control alternatives, with varying efficacies. This study assessed the efficacy of combining entomopathogenic fungal isolates (EPF) and a botanical compound for control of fungus gnat larvae. In a first series of bioassays, three selected fungal isolates *Metarhizium brunneum* (Cb15), *Beauveria bassiana* (EABb04/01-Tip) and one soil-borne endophytic fungus (*Acremonium strictum*) were applied at a rate of 1×10^7 spores/ml directly to two growing media with different constituents. 24 h post treatment (HPT), twenty fungus gnat larvae were released into each experimental cup and adult emergence was evaluated about two weeks later using yellow sticky cards. Larvae exposed to *M. brunneum* were more susceptible as compared to the other fungal strains; this EPF significantly reduced the emergence of adults. Larval mortality varied from 75% to 77% in each substrate. In a second series of bioassays, larvae were transferred into the substrate treated with a botanical compound, containing mentha oil. A higher percentage of fungus gnat adults emerged from larvae exposed to the botanical microcapsule (65%) than the control treatment, exhibiting an attractant effect for the larvae. Subsequent bioassays were set-up to assess the efficacy of *M. brunneum* and two biopesticides individually or in combination with the botanical compound aimed at reducing adult fungus gnat numbers. In this experiment, the EPF and two biopesticides (Neem Azal and Spinosad) were used at either the same rate mentioned above or the recommended application rate to the substrate treated with the botanical microcapsule. *M. brunneum* and the two biopesticides, when applied alone, were effective against fungus gnat larvae. The combination of these treatments with the botanical compound resulted in an additive effect as shown by the reduced number of adults emerging. Specifically, the combination of Spinosad and the botanical microcapsule significantly reduced the emergence of adults by 97% as compared to the Neem Azal and fungal treatments in combination with the botanical compound (90.5% and 84%, respectively). These results point to an “attract and kill” effect, which might efficiently reduce fungus gnat populations.

Effect of host plant species on the efficacy of entomopathogenic nematodes versus *Delia radicum*

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The cabbage root fly (*Delia radicum*) is considered as one of the most important cabbage pests in Europe and North America. Producers lack effective measures to control larvae of root flies since more and more chemical compounds with activity in the soil are phasing out. The following results are based on an experiment involving the tritrophic interaction between Brussels sprout (*Brassica oleracea* var. *gemmifera*), the cabbage root fly and entomopathogenic nematodes. Two different varieties of Brussels sprouts were tested, differing in their glucosinolate content. Nematode treatments include *Heterorhabditis bacteriophora*, *Steinernema feltiae* and a combination of both species. Efficacy was dependent on the plant variety, insect larval stage and the nematode species. Efficacy of entomopathogenic nematodes on the first larval stage reached 27% to 62%, whereas on the third larval stage, efficacy of 45% to 83% was recorded. The nematode efficacy was higher for the variety "Content" with higher glucosinolate content than for the variety "Esperal" with lower glucosinolate content. At the first larval stage, *Heterorhabditis bacteriophora* was the most effective nematode on the variety "Content". The results will build a basis for improvement of biocontrol strategies against the cabbage root fly with entomopathogenic nematodes.

***Tuta absoluta*: A new approach in biological pest control by granulovirus „Tutavir“ and *Steinernema*-nematodes in an operational tomato greenhouse**

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Infestations with tomato leaf miner *Tuta absoluta* increased in North Rhine Westphalia within the last few years. The pesticides Coragen (Chlorantraniliprole) and Spintor (Spinosad), both registered in Germany, gave no longer sufficient results. Tutavir, a new product by Andermatt Biocontrol/Switzerland containing the granulosis virus PhopGV, has recently been developed in a European project. In an experimentation conducted at Neurather Gärtner/Grevenbroich in cooperation with the Plant Protection Service, the following products were tested in summer 2018 in a 2,5 hectare tomato production greenhouse (‘Red Ruby II’) over a period of 2½ months:

- Tutavir (100ml/ha, first time: 200ml/ha; water: 1600 l/ha)
- entomopathogenic nematode *Steinernema feltiae* (160000 nem./m²; water: 2000 l/ha)
- Coragen (Chlorantraniliprole), (315 ml/ha; water: 1600 l/ha)
- and an untreated control

Each variant contained a surface of 2500 m² with four replications. The applications were conducted by a semiautomatic spraying robot: Tutavir and nematodes were sprayed once a week for 7 weeks, Coragen only once at the beginning of the trial. The number of little mines (L1+L2), big mines (L3+L4) and the number of damaged fruits were counted weekly on six separate levels. Additionally one pheromone trap per variant and replication was observed. Starting at a high infestation level (about 4 mines per plant), Tutavir decreased the number of mines and fruit damage, first slowly but continuously to less than 1 mine per plant. Nematodes had an effect slightly inferior to Tutavir, although it was observed that temperature and humidity have an important influence during and after spraying: at cloudy and colder summer days the effect was comparable to Tutavir, whereas it was not sufficient during hot summer days, although sprayed in the evening. The insecticide Coragen induced a decrease of the number of leaf mines beginning two weeks after the application for the next 4 weeks. Afterwards the infestation increased again and reached about 7 mines per plant, which is the same level as the untreated control. Regarding fruit damage, both Tutavir and nematodes showed a reduced infestation with 0,2, respectively 0,3 damaged fruits per plant, compared with 3,5 in the untreated control and Coragen. Concluding the Granulovirus Tutavir significantly showed the most efficient test results. This biological product could be an important contribution to resistance prevention and resistance management against *Tuta absoluta*. An application for registration in Germany (Art. 53) is in progress.

EcoOrchard - Boosting agro-biodiversity in European apple orchards

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The aim of the research project EcoOrchard was to develop appropriate strategies to promote functional agro-biodiversity (FAB) in organic apple production. In a pan-European approach research institutes, universities and advisory services of nine European countries have been involved. In these countries, a survey was conducted among advisors and farmers in 2015 to figure out the current status on knowledge and implementation regarding FAB and to identify specific differences depending on the national context. It became clear, that a majority of farmers were using methods to favor FAB at various levels, and that farmers were also interested in a 'monitoring-tool' to assess FAB in their orchards. Therefore, four easy-to-use methods have been tested and modified according to participative farmers in 2016 and 2017. A web-based platform (EBIO-Network: <http://ebionetwork.julius-kuehn.de>) has been created for sharing information on how to enhance functional biodiversity in the transnational context. It offers practical, comprehensive knowledge, eg. technical leaflets, general information and contact points, a voluntary stakeholder EU map, as well as a literature database on functional agro-biodiversity. On the scientific part, synchronised field trials have been performed at different sites in seven countries from 2015 on, where perennial flower strips were established in the inter-rows of the orchards. Natural enemies of pests like Syrphidae, Coccinellidae and parasitoids of codling moth are supposed to be promoted with these additional floral resources. To monitor the prevailing pest pressure as well as the state of biodiversity, various monitoring methods were applied in a standardized scheme: visual control, beating sampling, corrugated cardboard bands, sentinel prey cards and assessment of fruit damage. Based on this frequent monitoring, it has been shown that the tree rows within the flower strips covered a higher number of beneficials and less fruit damage compared to the control plots. In order to optimise the plant composition of flower strips, additional studies on effects of flowering plants on main pests and beneficials were carried out. Their requirements for food resources, particularly nectar and pollen, were investigated in adjoining laboratory and field experiments at JKI Damstadt, Institute for Biological Control.

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Establishment of predatory mites on undersown crops in hop cultivation

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The two-spotted spider mite *Tetranychus urticae* is one of the two major pests in hop cultivation. Conventional growers use acaricides to control spider mites, often in a preventive manner. In organic hops there is up to date no effective way of controlling spider mites. In vineyards or orchards, established populations of predatory mites solve this problem. In contrast, in a hop garden the entire plant biomass is removed from the field at harvest, and no habitat remains for predatory mites to overwinter in the field. We tested three different undersown crops in the driving lanes as hibernation quarters for beneficials: Tall fescue *Festuca arundinacea* already showed promising results in a previous project and provides not only habitat but also grass pollen as food for predatory mites in spring. Second, a grassland mixture of six legumes and eight grasses (e.g., *Alopecurus pratensis*, *Poa pratensis*, *Festuca pratensis*) was sown as food source for the mites and to create more attractive habitat for beneficials. Legumes are popular with organic farmers due to the biological nitrogen fixation. The third variant were strawberries as ligneous plants in the lanes, providing comparable hibernation quarters to vineyards or orchards without hampering the farmers regular works in the hop garden. The focus of the project is the native predatory mite *Typhlodromus pyri*, a well-established species in vineyards. We got grapevine cuttings in May during pruning of vineyards, cut them into small pieces and dispersed them in experimental hop gardens. We also tested purchasable predatory mites. In the first year of the project we used a mix of *Phytoseiulus persimilis* and *Amblyseius californicus* as well as *Amblyseius andersoni*. For those allochthonous predatory mites we also tested different ways of dispersal in the hop garden: On bean leaves, on vermiculite dispersed with mini air bug or strewn by hand, and in sachets at different stages of development. In 2018 we achieved only results regarding the different predatory mite species. We were not able to interpret the three undersown crops in the lanes as summer was unusual dry and hot and the different grasses did not grow well. So far, the predatory mites mix on bean leaves yielded best results and seemingly was most user-friendly for the growers. *Typhlodromus pyri* also performed quite well in one experimental field until heat and drought affected the predatory mites.

The dangerous life of the linden aphid *Eucallipterus tiliae* in the microcosm of a lime tree (*Tilia cordata*)

Urs Wyss¹

University of Kiel¹, Entofilm

This short movie (15 min.) first presents the only aphid species encountered in lime trees: the delicate red-eyed linden aphid *Eucallipterus tiliae*. Development from birth to winged adults and differences in honeydew discharge between juvenile and adult instars are shown. This introduction is followed by the presentation of natural enemies, with emphasis on predatory bugs, lacewing and hoverfly larvae as well as on two ladybirds species. *Coccinella septempunctata* was surprisingly never detected on the lime trees examined. Freshly hatched anthocorid bugs have to gain experience in order to overcome the defensive responses of attacked young aphids. Once paralysed by a sting, prey has no chance to escape. *Deraeocoris lutescens* is another predatory bug, commonly found on lime trees, especially towards the end of the season. Stalked lacewing eggs, arranged in a bunch were occasionally found. First instar juveniles, most likely belonging to *Pseudomallada prasina*, hatched more or less simultaneously, climbed down and started preying on nearby aphids. Several not identified hoverfly larvae colonized the tree. Aphids were caught and devoured by freshly hatched larvae when they adhered by coincidence to their sticky cuticle surface slime from which they were not able to disentangle themselves. Even a winged aphid was not able to detach the tip of one of its antennae, with the result that this aphid was finally consumed after a long lasting attempt to escape. Older hoverfly larvae usually responded immediately upon the slightest touch by an aphid. Among ladybirds, *Calvia decemguttata*, and *Harmonia axyridis* were the only two species encountered. In the case of *C. decemguttata*, recordings focused on the preying behaviour of last instar larvae and their transformation into the adult beetle. In the case of *Harmonia axyridis*, emphasis was placed on hatching, feeding behaviour of first instar larvae, including intraspecific competition and aphid response to released alarm pheromone. Intra guild predation and pupa emergence is shown for the last instar larva.

A parasitoid of stink bug eggs in a lime tree

Urs Wyss

Entofilm

The movie (8 min.duration) first shows several pentatomid bug species that live in a lime tree (*Tilia cordata*) and then a stink bug egg cluster of unknown species origin in the same tree. Embryo development inside these eggs is shown as well how first instar bug larvae hatch by using a structure that helps them to open a preformed lid of the hard egg shell. Hatched larvae stay for a rather long time on the eggs until they finally disperse. In another, at first sight identical egg cluster, a strange creature is seen to move around inside the egg in a pack-man like manner. Mysterious changes occur inside the eggs and finally, after about three days, it becomes evident for the first time that a parasitic wasp develops inside the eggs. Embryo development is especially fast in one single egg of the cluster, from which a male wasp emerges. Only females develop in all other eggs (11 -14). Hatching requires considerable energy as the wasps have to bite their way out through the hard lid of the egg shell. The early hatched single male, identified to be a species of the genus *Trissolcus*, has to wait outside the cluster for nearly two days until it can copulate with his emerging sisters. Mating in the absence of competing rivals is then a rather fast process.

Insect diversity and abundance in maize single crop and maize/bean polyculture

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Intercropping of maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) has a long tradition in Middle and South America. The bean plants can take up excess nitrogen and convert it into biomass, or - under N-shortage - supply part of their own N-demand through symbiotic nitrogen fixation. A denser canopy in maize-bean intercrops can suppress weed growth and decrease evaporation. The maize and bean plants can be harvested and silaged together and produce an energy-rich livestock feed or substrate for biogas production, with a higher protein content than maize only silage. Thanks to the continuous flowering of the bean plants for several months, an attractive food source for insects is introduced into the otherwise relatively species-poor maize fields. Due to these possible benefits, the implementation of maize/bean polycultures in European agriculture is currently tested and refined in several projects. A cropping system with an additional nutrient source for insects could lead to a subsequent diversification of the fauna, which could enhance the natural pest control by predators and parasitoids. To test this hypothesis, one flight interception trap was placed in a maize single crop and maize/bean intercropped field respectively at four sites in Germany (BW: Aulendorf, SL: Überherrn, NRW: Krefeld, Lengerich) during summer 2018. The traps were active for one to two weeks at three (Krefeld: four) collecting periods each - before, during and after the maize flowering. The biomass of the arthropod samples was determined and individuals were identified to group or order/family level. At the two sites in BW and SL the total arthropod biomass as well as the number of individuals collected was higher in the maize/bean polycultures compared to the maize single crop fields. More chalcid wasps (Chalcidoidea) were caught in the maize/bean fields at three locations. A slight trend to more bees (Apidae) in the maize/bean intercrop could also be observed. Furthermore, beetles (Coleoptera) and thrips (Thysanoptera) were more abundant in the intercropped fields. Because of the extremely hot and dry vegetation period in 2018, the bean growth was slowed down and fewer flowers were produced. In the fields in NRW, where the drought and its consequences were more severe compared to the south of Germany, also no positive influence of the intercropping on insect diversity and abundance could be observed. However, the trends seen in the groups of chalcid wasps, bees and beetles, and results from other studies that found an increase in insect diversity in intercropped systems, encourage further field trials with more sites and traps under different conditions.

**Innovative liquid core capsules for pest control based on the attract and kill principle:
a report on the first practical trials for Heteroptera bug control**

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A special type of liquid-core capsules was developed for pest control based on the "attract-and-kill" concept. The capsule consists of a liquid core and an outer shell that confers protection against environmental conditions and reduces dehydration. High quantities of volatile attractants can be integrated into the capsule to be released over a long period. Active substances and phagostimulants can be added to the liquid core. Exemplary, these capsules can be used to control the lygus bug (*Lygus rugulipennis*). The efficiency of the capsules was tested in the lab where the attracted bugs pierced the capsule shell with their proboscis and absorbed the active ingredients orally. The first greenhouse and field trials took place from June to October 2018. Capsules containing a mixture of attractants, but no pesticides, were applied to five different types of glue traps and the number of bugs trapped was recorded weekly. Glue panels without attractant capsules served as a control. *Lygus* sp. bugs were observed on all trial plots during the whole trial period but the number captured was low. Only in the migration phases, after the mating of the 1st generation and with the emergence of the 2nd generation, a higher number could be found on the traps. The mean number of trapped females was three times higher than in the control treatment. In the case of males, no differences were observed. Delta traps used were shown to be less suitable. In a further trial, the pest control potential was assessed on eggplants. Over a period of 3 months, capsules that contain a mixture of attractants and the agent acetamiprid were spread on leaves at 2-week intervals in a density of 200 capsules per m². Dead *Lygus* sp. could be observed regularly and directly beside the capsules. Individuals were also observed to tremble and die shortly after contact with the capsules. The plant infestation by the bug was not quantitatively assessed, but in contrast to the last year, no or only negligible damage was observed on the plants. The two to three plant treatments with acetamiprid and partly with flonicamid, which were the usual practice in the previous years, were not carried out. In conclusion, the basic functional principle could be confirmed in practical conditions; the attraction effect is rather limited to the surroundings of the capsules (presumably a few cm). More effective attractants and suitable biological agents will be developed in the future and field trials are required to determine the degree of efficiency.

Tachinid parasitoids: importance as biological control agents and remarks on their rearing

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In the order Diptera about 10,000 parasitoids belong to the Tachinidae family. With this number they represent only 20% of all insect parasitoids. However all tachinid flies are parasitoids in their larval stage, what makes them good candidates for biological control research. They have various parasitisation modes, which allow them to reach their hosts in different environments, such as hidden in vegetables or in the soil and due to that they are able to reduce the target pest populations. There are some successful examples for their application in biological control, but about their mass rearing less information is available. Worldwide, only a few working groups study Tachinid parasitic flies, one of them can be found at the Department of Agricultural and Food Sciences of University of Bologna. Here, the aim is to develop effective rearing technologies and to get more knowledge about them. *Exorista larvarum* (L.) (Diptera, Tachinidae) is a parasitoid generally known as an antagonist of lepidopterous defoliators in forestry and agriculture. This beneficial entomophagous insect can be easily reared *in vivo* and *in vitro* on artificial media. The *in vivo* rearing technique is generally performed by adopting a factitious host, such as *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Storage at low temperatures is a useful technique to extend insects' developmental time and thus elongate their shelf-life. Immobile stages, including puparia, are usually more tolerant to the cold exposure. In the present study, the effects of storage at 15 °C (for four weeks) on *in vivo* produced *E. larvarum* puparia were tested and compared with puparia stored at 26 °C. The results showed some decrease in adult quality parameters, namely longevity and fecundity, nonetheless, the storage treatment (15 °C) proved to be effective for extending the parasitoid development. According to the different purposes of insects' mass-rearing, a certain reduction in quality can be accepted in favour of a more flexible rearing schedule.

It's never too late, but sometimes too early – an exceptionally long mating reluctance period in *Bracon brevicornis* (Hymenoptera: Braconidae)

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The parasitic wasp *Bracon (Habrobracon) brevicornis* WESMAEL (Hymenoptera: Braconidae) is used as a natural enemy within biological pest control strategies, in particular against Lepidopteran pest species of the families Pyralidae and Noctuidae. Under normal rearing conditions an excessive number of male *B. brevicornis* wasps were observed within a relatively short time, which can lead to the population's extinction in only a few generations. This gregarious larval parasitoid is known to have a sex determination system which, particularly under inbreeding conditions, results in an increased number of infertile sons at the cost of fertile daughters. Due to individuals of this wasp species not being able to distinguish between a related and non-related mating partner, knowledge on inbreeding avoidance become a much more important factor for the preservation of a population. We found female adult *B. brevicornis* wasps to have an exceptionally long and gender-specific mating reluctance, which occurs only at an age of 24-48h after hatching from pupae. We analysed the latency period (emergence – readiness to mate) for two different populations of *B. brevicornis*, and within two subsequent generations. Despite this behavioural latency, our results show that younger wasps are not physiologically limited and could indeed mate successfully directly after emergence. When analysing the variability, we found differences in the female partner acceptance and a cross-generational correlations, which suggests a heritable mating behaviour. We conclude that the observed and relatively long reluctance period compensates the incapacity to identify the degree of kinship of a potential mating partner in this species, thereby supporting inbreeding avoidance. To establish new populations, and for commercial mass rearing of *B. brevicornis*, our data suggest methods, which avoid or exclude mating within the first 48h after hatching from pupae.

Keeping genetic variation in a laboratory population

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Laboratory or mass rearing of insect parasitoids rearing represents a challenge to keep variability within populations. This occurs especially in species that suffer from inbreeding depression, i.e. many Braconids, and it is vital to keep a certain minimum of genetic variation. We are working with the parasitoid wasp *Bracon (Habrobracon) brevicornis* Wesmeal (Hymenoptera: Braconidae), which is used as a control agent of several Lepidopteran pest species. We have combined four laboratory populations, which were based on only a few foundress females and had been collected from the field in Germany and France, into a new mixed line, using a round-robin mating approach. We will explain the establishment of this mixed population and the protocol that is used to retain genetic variation. We have also analysed the four base populations and the newly created mixed line in terms of behavioral variation in a life history trait, i.e. clutch size decisions of virgin females. We also estimated genetic variation by comparing inbreeding effects in inbred and randomly mated wasps. Our results show the potential of our rearing method for retaining genetic variation in laboratory populations.