

Report on the 35th Annual Meeting of the Working Group “Beneficial Arthropods and Entomopathogenic Nematodes”



After one year of “diapause”, our Working Group “Beneficial Arthropods and Entomopathogenic Nematodes” of DPG and DGaaE met on 28th and 29th November 2017 - this time at Julius Kühn-Institute in Berlin-Dahlem. The organization was in the hands of Dr. Peggy Marx, Julius Kühn Institute, Department of Ecological Chemistry, Plant Analysis and Stored Product Protection, leading to a very smooth, comfortable and “sunny” atmosphere (see group photo). We were happy to welcome participants from universities, research institutes, plant protection extension services and biocontrol companies. Special thanks to our retired, but still very busy colleagues Dr. Barbara Baier and Prof. Dr. Bernd Freier for joining our group this time! Topics of the contributions covered new developments in production and use of invertebrate biological control agents, side-effects of plant protection products on beneficials, challenges in out-door plant protection like in forestry and hop cultivation, but also basic research on host location mechanisms or climate adaptation of particular organisms. Bernd Wührer (AMW Nützlinge GmbH, Pfungstadt) reported on newest developments presented on the annual meeting of the biocontrol industry (ABIM, 23 – 25 October 2017, Basel: <http://www.abim.ch/archive.html#c1876>). Some contributions were already published in scientific journals (von Houten et al. (2017): *Ways to improve biocontrol of tomato russet mites using predatory mites*. IOBC-WPRS Bulletin Vol. 124, 189-194; Hübner et al. (2017): *Effect of entomopathogenic nematodes on different developmental stages of Drosophila suzukii in and outside fruits*. BioControl 62, 69–680). Short abstracts of other presentations given by participants of our meeting are compiled in this report. We would like to thank all for their attendance and lively discussions during the meeting and the evening spent at the “Eierschale” in Berlin-Dahlem. The next meeting will be organized hopefully in 2018 – this time linked to the “Arbeitstagung Biologische Schädlingsbekämpfung” – and held at the Botanika in Bremen from November 27th to 28th 2018. Please expect our invitation in summer 2018.

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

BIOCOMES – Results of a European project on the development of biological plant protection products to control insects

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The availability of sufficient biological control products is important for an effective IPM strategy. Biological control alternatives against a range of important pests and pathogens – causing high economic losses to agriculture and forestry – are not or not sufficiently available at this moment. The EU gave a major boost to the biological control market by co-financing the BIOCOMES project. At the end of this project in November 2017 BIOCOMES partners have developed several new biological control products to control a number of important pests and diseases in agriculture, horticulture and forestry. Some of the BIOCOMES products will be available at the end of 2017. Others are ready for registration and available on the market thereafter. For more information please consult the webpage www.biocomes.eu, which also provides interviews with the partner scientists reporting the success of the project.

Online Database on Beneficial Arthropods-Friendly Plant Protection

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Preserving populations of beneficial arthropods (e.g. predatory mites or ladybirds) as antagonists to harmful arthropods (e.g. spider mites or aphids) is an important part of plant protection strategies. In addition to the importance of the beneficials as a natural, restrictive factor to harmful arthropods, they constitute a vital part of agricultural ecosystems and contribute to the preservation and sustainable utilization of the biological diversity in agriculture. According to European and German national regulations, the Department of Ecological Chemistry, Plant Analysis and Stored Product Protection of the Federal Research Centre for Cultivated Plants (JKI) evaluates the effect of plant protection products (PPP) on beneficial arthropods and provides a position statement for every PPP subject to the authorisation process. Therefore, the effect of PPP on beneficial arthropods in horticultural or agricultural areas and forestry are scientifically evaluated. As a result, a species-specific PPP classification concerning their effects on beneficial arthropods is provided in three categories called „not harmful“, „slightly harmful“ or „harmful“. This risk assessment of plant protection products should enable a selection of plant protection products within the integrated pest management, which are harmless to beneficial arthropods. The species-specified labelling of the plant protection products is, however, assigned by the Federal Office of Consumer Protection and Food Safety (BVL) as a group-specific summary like „Populations of Relevant Predatory Mites and Spiders“ or “Populations of Relevant Beneficial Insects“. To facilitate the access to a species-specific evaluation of each plant protection product, an online database will be developed under the homepage of the JKI, covering the topic of beneficial arthropods-friendly plant protection. The database will provide further information on the procedure of the classification as well as relevant beneficial arthropods and their importance for the agricultural system. Professional users of plant protection products, crop consultants and allotment gardeners as well as the general public will be the target groups of this database.

Comparative efficacy of four entomopathogenic nematode isolates against the Tomato Leafminer *Tuta absoluta* in laboratory leaf bioassay

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The tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) is a serious insect pest of tomatoes. Entomopathogenic nematodes are one of the promising biocontrol agents against this pest. In this study, laboratory bioassays were conducted to investigate the efficacy of four entomopathogenic nematode isolates against *T. absoluta* larvae. The nematode isolates were *Steinernema carpocapsae* (Weiser) [BA2 isolate], *S. abbasi* Elawad, Ahmad & Reid, *S. feltiae* (Filipjev), and *Steinernema* sp. [J7 isolate] and originate from different regions (Egypt, The Sultanate of Oman, Germany and Germany, respectively). The nematodes face some challenges because of the special habitat of *T. absoluta* larvae, which feed and develop inside galleries they make in leaves. These challenges for nematodes are to locate the mines on the leaf surface and to penetrate into the mines and infect the host inside. Based on these particularities, a leaf bioassay was developed to evaluate the efficacy in a comparable manner. The different nematode isolates were applied in different concentrations (15, 30, 60, 125, 250, 500, and 1000 IJs/ml) against the 4th instar larvae in tomato leaflets. The results were used to calculate median lethal concentrations causing 50% larval mortality (LC₅₀) and their confidential limits for the four isolates. The values of LC₅₀ were 44 IJs/ml for *S. carpocapsae* BA2, 87 IJs/ml for *S. abbasi*, 113 IJs/ml for *S. feltiae*, and 103 IJs/ml for *Steinernema* sp. J7. Based on these values, *S. carpocapsae* BA2 was clearly the most virulent isolate in this study. As a next step, semi-field studies considering efficacy of this isolate under more natural conditions and also focusing on best formulation and application techniques are underway.

Control of *Agriotes* ssp. by the entomopathogenic fungus *Metarhizium brunneum* (Attracap®) in hops

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The larvae of *Agriotes* ssp. (wireworms) are 5 to 30 mm long, yellowish colored and live for one to three years in the soil. In the permanent crop hop (*Humulus lupulus*) the most common species are *A. lineatus* and *A. obscurus*. The main damage on hops occurs in early April, when the growing phase of hop is starting after the winter dormant phase and the shoots are very small and fine. Wireworms are in the soil surrounding the roots and are attracted by their CO₂ emission. The feeding damage on roots could lead in the worst case to a necrosis of roots and shoots. However, the actual impact of wireworms on hop is negligibly low because of chemical insecticide use in early spring.

To reduce the amount of chemicals in plant production the efficacy of an alternative control agent, like the entomopathogenic fungus *Metarhizium brunneum*, was investigated. Attracap® (Biocare) uses the method of “Kill-and-Attract” with a combination of a CO₂ producing yeast, as an alternative attractants and *Metarhizium brunneum*, which infects and kills the wireworms. This bioinsecticide is formulated as a granule and was applied in a field trial in the Hallertau in May 2017 with a specific application device to apply granules directly into the soil. A treatment of 30 kg/ha Attracap® was compared to an untreated control. The first assessment was performed in September 2017 with potatoes, which were planted between the hop plant rows, as an indicator plant. The assessment of hop root damage during the growing season is not possible without damaging the root stock. The results indicated a significantly reduced feeding damage of 50.1% in the treated area compared to the non-treated plots with 67.4% (p-value 0,047, t-test). The reduced feeding damage on potatoes is indicating a successful establishment of the entomopathogenic fungi in the hop garden and probably also a positive effect on hop roots. The impact of Attracap® on the hop plants will be assessed in spring 2018 by assessing the feeding damage on the cut hop rhizomes. In order to achieve a long-lasting and stronger efficacy of *Metarhizium brunneum* against wireworms in hops a reapplication for two or more seasons is discussed.

Chemical diversity in plant/beneficial arthropod interactions: Tool or obstacle?

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In many European cropping systems key parasitoids or predators of arthropod pests are sufficiently widespread and abundant for biocontrol of the pest organisms. However, their efficacy is often not high enough to warrant a substantial reduction of their hosts or prey - and of yield losses. Can plant chemical diversity be used as a tool to enhance the efficacy of beneficial arthropods? What kind of research is needed to answer this question? Plant - arthropod interactions have evolved in chemically complex environments, formed primarily by host and non-host plants. Natural enemies of crop pests have evolved physiological and behavioral mechanisms to cope with or even to utilize the chemical diversity of the crop environment for successful host finding. Also in agroecosystems, plant constituents fulfill a multitude of biological functions and determine the relationships between crops, pests and beneficial arthropods from the level of molecules to their function in the ecosystem and the agricultural landscape. In conservation biological control, strategies for conservation and enhancement of parasitoid or predator efficacy need to be developed or improved that involve a positive effect of chemical diversity on beneficial arthropods. One option to achieve this is to adjust the composition of field margins or flowering areas in a way that they are supporting functional populations of natural enemies of nearby crop pests. While commercial seed mixtures for birds, bees and butterflies exist, there are hardly any mixtures available supporting biocontrol. Seed mixtures for field margins or flowering areas including non-host plants are needed that support host search, maintenance and survival of beneficial arthropods and deliver ecosystem functioning and ecosystem services by providing the required chemical diversity. To achieve this a) the diversity of plant compounds from selected field margin or flowering area plants need to be investigated and b) the scent diversity of the entire flowering area needs to be determined and compared with overall arthropod biodiversity and specific multitrophic interactions. At the same time, it should be investigated how the composition of primary and secondary plant compounds varies depending on the abiotic environment and affects the arthropod communities in terms of plant quality, resistance and stress tolerance on the arable land and in the neighboring flowering areas. This knowledge will allow deciding if chemical diversity in plant/beneficial arthropod interactions can be applied as biocontrol tool.

Spider mite management in hop cultivation: state of play, ten years later

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Spider mite (*Tetranychus urticae*) control in hop cultivation and especially in organic hops, by the use of predatory mites has been investigated in the Bavarian Hop Research Center since the early 1990s. However, it was only in 2007 when it was first shown in a large trial that spider mites can be effectively controlled by release of predatory mites (*N. californicus* and *P. persimilis*). Since then, numerous trials and investigations were executed to advance this biological control method in the specialty crop hops. During 2016 and 2017, an experiment was laid out in a hop garden that had suffered from severe spider mite damage associated to trials without acaricide use in 2015. On untreated hop plants, which had suffered a total damage in 2015, one year later the spider mite population collapsed between the 18th of July and the 1st of August from 50-75 individuals per leaf to nil and did not recover until harvest. Plots with released predatory mites (*T. pyri*) did not vary significantly from control plots in 2016. In 2017, the second year after a total damage, the mix of *N. californicus* and *P. persimilis* was released again and yielded highly satisfying spider mite control. In release plots spider mite numbers never surpassed an average of 10 individuals per leaf until harvest, when they reached a maximum of 15 mites per leaf, and were significantly lower than in the control plots during the last five vegetation weeks until harvest. The untreated control plots exhibited a stable spider mite population increase to almost 100 individuals per leaf at harvest, which did not yet lead to yield or quality loss – although this value was admittedly close to a slight damage. In a nutshell, these 2-year trials proved that heavy spider mite damage in a hop garden in one year does not necessarily cause damage in the following year, but that the damage obviously can trigger a kind of resistance mechanism in the particular plant induced by a previously experienced infestation.

Innovative liquid core capsules for biotechnological processes – possibility for mass rearing beneficial insects and for pest control

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The conventional method for mass rearing of beneficials is based on a controlled growing of pests on respective plants. Therefore, plant cultivation is a major requirement, usually done in a glass house, taking a huge amount of energy and time and a high process risk. In order to optimize the whole process, the use of artificial nutritions could be an alternative. So far, there has not been any suitable formulation for the large-scale production of liquid diets to feed beneficials with sucking mouth parts. This was achieved with the development of special microcapsules, so-called liquid core capsules. The liquid diet forms the capsules core. The outer shell adds protection against environmental influences and reduces the dehydration dramatically, but on the other hand enables the penetration with the mouth parts of the beneficials. The production method is based on two steps: first a shape giving process, which traps the liquid diet inside a crosslinked alginate hydrogel-shell. Secondly, the shell is coated with a functional outer shell by spray-coating in a fluidized bed system. The use of special additives allows the control of diverse parameters, like shell size, stiffness or surface loading and is a requirement for successful coating in the fluidized bed. This way of feeding the beneficials is used in our internal mass rearing of lacewing larvae (*Chrysoperla carnea*). Throughout the whole development of the larvae our experience is very good. The development speed and fitness of the insects is comparable to the classical rearing by feeding with aphids. Possible changes in prey preference or negative impact to the fitness of the insects could not be observed. Beside the described liquid core capsule, a second capsule was developed for the “attract and kill” method. High quantities of highly volatile attractants can be integrated into the envelope and released over a very long period of time. Inside the liquid core an active substance and a phagostimulant are added. These capsules are, for instance, used to control the population of *Lygus* bugs (*Lygus rugulipennis*). The attractants are specific to *Lygus* bugs and have a higher affinity as compared to the host plant. After contact, they pierce the capsule shell with their proboscis and absorb the active ingredients orally. The advantages of the capsule are no contact of active agent with the crop, no waiting period between treatment and harvesting, exact dosing and reduced dosing of active agents and high selectivity to pest insects. It can also be used for active substances that do not have any effect on sucking pests in conventional application (leaf applications) and has the ability of mechanical application. Although testing of the practical suitability is still under way, the basic suitability could be clearly demonstrated in previous laboratory tests.

Natural product chemistry in stored product protection: How the larval parasitoid *Holepyris sylvanidis* locates its host, the confused flour beetle *Tribolium confusum*, by using specific volatile and non-volatile compounds.

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Parasitic Hymenoptera play an important role in multitrophic interactions as antagonists of arthropod hosts and contribute significantly to the stability of many ecosystems. Since parasitoids are able to control and regulate the population density of their host species they can be used as biological control agents against many herbivorous pest insects. In the field of integrated stored product protection it has also been shown that application of natural enemies of specific pests represents a promising approach and useful addition to conventional chemical and physical control measures. Nevertheless, to establish a successful biological control of insects infesting stored food it is essential to deepen and broaden our knowledge of the factors that drive the parasitoid's host location process. Essentially, these factors are volatile and non-volatile chemical compounds which are directly or indirectly associated with the host. In the present study we investigated, which naturally occurring odors are used by the larval ectoparasitoid *Holepyris sylvanidis* (Hymenoptera, Bethylidae) to find its host. *H. sylvanidis* has been described to parasitize different stored product pest beetles, but its preferred host are larvae of the confused flour beetle *Tribolium confusum* (Coleoptera, Tenebrionidae), a major pest of simply processed plant products. Headspace volatiles of the host-substrate-complex were chemically analyzed by GC-MS. The parasitoid's antennal responses to identified compounds were electrophysiologically measured by GC-EAD and EAG. Behavioral tests using a 4-field-olfactometer revealed that female *H. sylvanidis* are strongly attracted to a mix of specifically host-associated components of the *T. confusum* fecal odor in combination with volatiles ubiquitously present in the host's habitat enriched with wheat grist. The results suggested that a blend of host-specific compounds and habitat odor may serve the parasitoid as long-range attractant for host location from a distance. Generally, when parasitoids are in close proximity to their hosts, less volatile host-related chemicals are important for host finding success. Therefore, we studied the short-range attraction of *H. sylvanidis* and demonstrated that female parasitoids, at first, randomly search for contact host kairomones released from the host's cuticle onto the substrate. GC-MS analysis of host larval extracts and subsequent contact and trail following bioassays indicated that perception of fresh host larval cuticular hydrocarbons elicit trail following behavior in the parasitoid and also mediate host recognition. Hence, the host searching strategy of *H. sylvanidis* switched from a biased random search to directional responses and resulted in successful host location of *T. confusum*.

Fürstenau, B., Adler, C., Schulz, H., Hilker, M. (2016) Host habitat volatiles enhance the olfactory response of the larval parasitoid *Holepyris sylvanidis* to specifically host-associated cues. *Chem Sens* 41:611-621.

Fürstenau, B. and Hilker, M. (2017) Cuticular hydrocarbons of *Tribolium confusum* larvae mediate trail following and host recognition in the ectoparasitoid *Holepyris sylvanidis*. J Chem Ecol 43:858-868

Investigation on behavioral response of *Harmonia axyridis* to heat stress using EthoVision XT 8.0

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Temperature is one of the most important driving forces of ecological processes in insects. The projected increase of heat periods in Central Europe raises the question of how beneficial arthropods, such as coccinellids, respond to heat stress. Invasive *Harmonia axyridis* (PALLAS) (Coleoptera, Coccinellidae) has rapidly spread over Germany during the last years. The species is assumed to have a high potential to adapt to various environmental conditions and to compete with native species. Therefore, a lot of studies try to quantify their traits, performance and invasive potential under local environmental conditions. Our recent investigations focused on the response of *H. axyridis* to heat stress periods. A set of biochemical, ecological and behavioral test systems was designed. Video tracking was chosen to assess potential changes in activity traits of *H. axyridis* under heat stress. Within a climate chamber experiment 10 day old adult *H. axyridis* females reared at normal temperature conditions (mean: 17.8 °C) were treated with heat stress (5 hours at 37 °C) or without heat stress (5 h at 17.8 °C) as well as with or without moistened sponge inside aerated Petri dishes (14.5 cm diameter). Video tracking was conducted using the software EthovisionXT 8.0. The software proved to be a suitable tool to assess behavioral responses of *H. axyridis* to heat stress within the given experimental setting. The analysis was performed using the method “differencing” and an appropriate sample rate of 1.870 pictures per second. At heat stress significantly less activity duration and frequency of the individuals was recorded. A strong tendency for stress-related behavioral responses, such as jumpy motions, high maximum speeds and pathways at the edges of the experimental arenas were also observed. The presence of the moistened sponge had no significant effect within one temperature treatment but heat stressed individuals showed significantly longer stays at the sponge. Within the presented study video-tracking turned out to be a promising approach to analyze heat stress responses of coccinellids. Further tests will focus on a comparison between native and invasive species and contribute to develop the experimental setting.

Deutsche Phytomedizinische Gesellschaft e. V. & Deutsche Gesellschaft für allgemeine und angewandte Entomologie e. V.

35. Tagung des AK: „Nutzarthropoden und Entomopathogene Nematoden“

28.11. und 29.11.2017, **Julius Kühn-Institut Berlin-Dahlem, Königin-Luise-Straße 19, Berlin, im großen Sitzungssaal A 300**

PROGRAMM (Stand 24.11.17)

Dienstag, 28.November 2017

- 14:00 Uhr Begrüßung und Eröffnung der Tagung
*Dr. P. Marx, Julius Kühn-Institut Berlin Dahlem,
Dr. A. Herz & Prof. Dr. R.-U. Ehlers, Vorsitz AK*
- 14:20 Uhr Biocomes: Ergebnisse des europäischen Forschungsprojekts zur Entwicklung von biologischen Pflanzenschutzmitteln - Mittel gegen Insekten
Ehlers, R.-U., e-nema GmbH
- 14:40 Uhr Erstellung eines Wissensportals zu Auswirkungen von Pflanzenschutzmitteln auf Nutzorganismen
Marx P., Julius Kühn-Institut Berlin
- 15:00 Uhr Wirkungsvergleich von NeemAzal-T/S, Neemgranulat und B.t.i. gegen die Trauermücke *Bradysia impatiens* Johannsen 1912 (Diptera: Sciaridae) mit zwei unterschiedlichen Testmethoden
Kühne, S., Julius Kühn-Institut Kleinmachnow
- 15:20 Uhr Comparative efficacy of four entomopathogenic nematode isolates against the tomato leafminer *Tuta absoluta* in laboratory leaf bioassay
Abonaem, M., Julius Kühn-Institut Darmstadt
- 15:40 Uhr Sind entomopathogene Nematoden doch ein Mittel zur Bekämpfung der Kirschessigfliege?
Herz, Annette, JKI Darmstadt
- 16:00 Uhr Kaffeepause**
- 16:30 Uhr Poster: Einfluss von Untersaaten auf das Nützlingsvorkommen in der Baumschulwirtschaft
Preuss, S., Balder, H., Büttner, C., Beuth Hochschule für Technik
- 16:40 Uhr Kontrolle des Drahtwurms (*Agriotes* ssp.) mittels *Metarhizium brunneum* (Attracap(R)) in der Kultur Hopfen.
Wolf, S., LfL-IPZ Hopfenforschungszentrum
- 17:00 Uhr Naturnaher Waldschutz - Möglichkeiten und Erfordernisse des Einsatzes von Nutzarthropoden gegen Blätter und Nadeln fressende Insekten in Wäldern
Müller, M., Technische Universität Dresden
- 17:20 Uhr Methodik von Vergleichsfängen mit Insektenfallen und Insektenlockstoffen
Müller, M., Technische Universität Dresden
- 17:40 Uhr Chemische Diversität in Pflanzen - Nutzarthropoden Interaktionen: Werkzeug oder Hindernis
Meiners, T., JKI Berlin
- 18:00 Uhr Nutzarthropoden-AK-Quo vadis? - Denkanstöße für den Gemeinsamen Abend.
Herz, A., JKI Darmstadt

ab 19:00 Uhr: Gemeinsames Abendessen in der „Eierschale“, Podbielskiallee 50, 14195 Berlin-Zehlendorf, <http://www.berliner-eierschale.de>

Mittwoch, 29. November 2017

- 09:00 Uhr Ways to improve biocontrol of tomato russet mites using predatory mites
Knapp, M., Koppert Biological Systems
- 09:20 Uhr Einsatz und Etablierung von Raubmilben zur Spinnmilbenbekämpfung im Hopfenanbau: Stand der Dinge
Weihrauch, F., LfL-IPZ Hopfenforschungszentrum
- 09:40 Uhr Neuartige Flüssigkernkapseln für biotechnologische Verfahren - Möglichkeiten zur Nützlingsvermehrung und Schädlingsbekämpfung
Rademacher, J., Katz Biotech AG
- 10:00 Uhr Praxisbericht zur Anwendung biologischer Verfahren in historischen Garten an Kübelpflanzen
Jäckel, B., Pflanzenschutzamt Berlin
- 10:20 Uhr Kaffeepause**
- 10:50 Uhr Naturstoffchemie im Vorratslager: Wie der Larvalparasitoid *Holepyris sylvanidis* seinen Wirt, den Reismehlkäfer *Tribolium confusum* anhand von spezifischen flüchtigen sowie nicht-flüchtigen Verbindungen findet und erkennt
Fürstenau, B., JKI Berlin
- 11:10 Uhr Verhaltensanalyse zum Einfluss von Hitzestress auf *Harmonia axyridis* mit EthoVision XT8.
Krengel, S., JKI Kleinmachnow
- 11:30 Uhr Ausstattung von Baumschulware mit Nützlingen - nur eine Vision?
Balder, H., Beuth Hochschule für Technik, Berlin
- 11:50 Uhr Bericht von der ABIM 2017
Wührer, B., AMW Nützlinge GmbH
- 12:10 Uhr nächste Tagung wann und wo?

12:15 Uhr: Ende der 35. Tagung des AK