Report on the 38th Annual Meeting of the Working Group "Beneficial Arthropods and Entomopathogenic Nematodes", 24. – 25. November 2022, Schwentinental, Germany

After two years of Corona break, we were pleased to organize our 38th meeting of the working group "Beneficial organisms and entomopathogenic nematodes" at Schwentinental near Kiel from 24. to 25. November 2022 – fortunately again with fruitful and nice discussions and get-togethers during the coffee breaks and the evening event. We were very happy that e-nema GmbH invited us to their place in Northern Germany and welcomed us very kindly in their facilities. The meeting included 12 lectures, a poster session and a delightful contribution by Prof. Urs Wyss with his impressive film about "The dangerous life of the lime-tree aphid *Eucallipterus tiliae*". On the occasion of the 25th anniversary of e-nema GmbH, it was a special event to see the production facilities for entomopathogenic nematodes and various microorganisms that have "grown" in the course of the company's history, including the fermenters "Asterix" (capacity ???) and "Obelix" (capacity ???l).

The success story of the application of the minute parasitic wasp Trichogramma against the European Corn Borer by the company BIOCARE GmbH was presented by the former managing director Wilhelm Beitzen-Heineke, also on the occasion of the 25th anniversary.

Several new developments were reported, including a novel, practical gel formulation of entomopathogenic nematodes and results of several PhD projects on beneficial insects as biological control agents against various insect pests in arable, vegetable and fruit crops. Findings on possible genetic improvement through selective breeding were presented as well.

We would like to thank all the participants, especially those who provided us with short abstracts of their papers, which are summarized in this report. In addition, you will find three abstracts from our last meeting before Corona, organized by Dr. M. Zunker at LTZ Augustenberg near Karlsruhe, Germany, November 26-27, 2019.

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

25 Jahre e-nema GmbH - Biotechnische Produktion von Pflanzenschutzmitteln

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Die Biotechnologie gehört derzeit zu den innovativsten Wissenschafts- und Wirtschaftszweigen. Dabei umfasst der Begriff einen weiten Bereich, von der Stammzellenforschung über die genetische Modifizierung von Pflanzen, Bakterien und Tieren bis hin zur Produktion von Bioethanol. Traditionelle biotechnologische Verfahren sind beispielsweise das Bierbrauen oder die Nutzung der Backhefe.

Die e-nema GmbH hat sich auf die industrielle Produktion von Mikroorganismen und Nematoden in Bioreaktoren spezialisiert. Angefangen haben wir 1997 mit der Produktion von Heterorhabditis bacteriophora, einem insektenpathogenen Fadenwurm, in einem 500 Liter Bioreaktor. Inzwischen produzieren wir regelmäßig verschiedene Nematoden- und Bakterienarten sowie diverse Hefen und Pilze in zahlreichen Bioreaktoren mit Kapazitäten bis zu 100.000 Liter.

Aus den Organismen werden verschiedenste Produkte hergestellt. In biologischen Pflanzenschutzmitteln sind meist die lebenden Organismen die aktive Substanz. Sie werden durch entsprechende Maßnahmen von der Fermentationsbrühe getrennt und in lagerfähige Produkte überführt. In der Biokatalyse geht es dagegen um bestimmte Substanzen, die die Mikroorganismen produzieren. Hier müssen die Bakterien bzw. Pilze also aus dem Endprodukt herausgehalten werden. Es gibt eine unüberschaubare Vielfalt an Substanzen, die auf diesem Wege produziert werden können. Einige davon können auch chemisch synthetisiert werden; oftmals ist der biokatalytische Weg aber günstiger. Ein kurzer Abriss über die 25-jährige Geschichte soll einführen in die Führung durch die Anlagen der Firma.

Heterorhabditis bacteriophora: An excellent model for genetic improvement of biocontrol traits

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More than 150 different invertebrates are currently used in biological control of insect pests. For breeding insects and mites, producers of invertebrate biocontrol agents largely depend on sampling and characterising natural populations. Entomopathogenic nematodes (EPN), especially *Heterorhabditis bacteriophora* are different. The contribution will describe the relevant biological peculiarities and shortly introduce into techniques to show why this nematode is an excellent model for genetic improvement. *Heterorhabditis bacteriophora* biology permits production of inbred lines through self-fertilisation by the hermaphrodite and production of hybrids through crosses of second generation amphimictic adults. The genetic pool can be preserved by storage in liquid nitrogen. Mass production is done in industrial scale bioreactors in liquid culture but can also be done in small scale polyxenic cultures. EPN have a short life cycle allowing rapid progress by genetic selection. Several traits have been improved, e.g. reproduction, longevity, field persistence and stress resistance to heat, desiccation, reactive oxygen species and nematicides. EPN can be subjected to EMS mutagenesis. Genetic selection is successful if the hertibailty of the trait is high, however the progress is easily lost due to outcrossing during mass production. As *Heterorhabditis* spp. are unable to mate in liquid media, reproduction is only through self-fertilising hermaphrodites. The use of well characterised inbred lines can overcome problems of trait deterioration when production is done in liquid media. A large pool of molecular genetic information and tools are available to support breeding of heterorhabditid biocontrol agents and a huge pool of sequence data are available.

Broad phenotyping of DJ-recovery in *Heterorhabditis bacteriophora* using highly homozygous mutants and WT-inbred lines

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The entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* is an effective biological control agent against insect pests. The dauer juvenile (DJ) is in charge of seeking for new hosts and carrying its symbiotic bacteria (*Photorhabdus* spp.). Upon DJ infection to the insect, DJs reach the haemocoel where their further development is triggered by insect signals (event called DJ-recovery). This event is crucial for the reproductive success in EPN. In commercial production, DJs recover upon contact with pre-cultured *Photorhabdus* cells and the major regulators of the triggering interaction are not well understood. To enhance the understanding of the genetic mechanism regulating DJ-recovery in *H. bacteriophora*, we have combined pheno- and geno- typing approaches. A collection of > 100 EMS-mutant and 48 wild-type (WT) inbred lines was produced. All lines were evaluated for recovery induced by cell-free bacterial supernatants. As complementary traits, the DJ survival (MTS_{50}) under oxidative stress and virulence against mealworm (*Tenebrio molitor*) were as well evaluated. The DJ-recovery in bacterial supernatant and monoxenic liquid cultures resulted strongly correlated. The DJs of mutant lines showed highly contrasting recovery (3.9 - 76.6 %), virulence (19.2 - 68.1 DJs/larvae), and MTS_{50} (10 - 151 hours). Parallel to phenotyping, we carried out genotyping by sequencing (GBS) in all mutant and WT lines. At least 13 out of 500 evaluated single nucleotide polymorphisms (SNPs) showed potential association to DJ-recovery. The present pheno- and geno-typic results are the basis for future physiologic and molecular approaches to identify regulatory elements in complex quantitative traits in *H. bacteriophora*.

Novel capsule formulation for nematodes - effects and possibilities for application

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A novel and patented capsule formulation for entomopathogenic nematodes (EPN) was presented. The EPNs are contained in a liquid core consisting of an emulsion of vegetable oil with water, which is enclosed in a shell of calcium alginate. The emulsion has positive effects on the EPN. After 14 days of storage at 22 °C and 100 %RH, 80 % and after 21 days 10 % of the nematodes were still alive. In the capsules without oil, no living animals were found after 14 days. Cooling increases the longevity. After 12 weeks of storage at 8 °C, an average of 70 % of the EPNs were still alive in the capsules with oil. Studies on the efficacy of the capsule formulation on larvae of the meal worm beetle (*Tenebrio molitor*) were carried out in a standardised test system. The system is established in the quality control of EPN and the results are transferable to plant pests such as fungus gnat larvae. Compared to a conventional application of *Steinernema feltiae* with water, the efficiency of the capsules was between 20 % and 30 % higher over time. The period of effect was also extended by about three weeks to over eight weeks. Similar positive effects of encapsulation were also observed with other nematode species. However, there were clear differences between the species in the course of efficacy at comparable doses. Three weeks after treatment, the initial efficacy was reduced by only 4 % in *Steinernema carpocapsae*, 23 % in *S. feltiae* and 52 % in *Heterorhabditis bacteriophora*. After four weeks, the efficiency was reduced by 17 % in *S.c.*, by 19 % in *S.f.* and by 62 % in *H.b.*

The release of EPNs from the capsules occurs only after mixing into a substrate and with a time delay. Observations have shown that nematodes present in the substrate penetrate the capsules from the outside and thus the capsule wall also becomes permeable to EPN. Microorganisms also decompose the capsules in one to three weeks, depending on their activity. There is no release in substrates that were sterilised before application.

Due to the delayed onset of efficacy and the long-term effect, the capsules can be used prophylactically and are very suitable for initial treatment. The manufacturing process is suitable for mass production and with the product nemaplus®depot, a capsule formulation for the control of fungus gnats is already available. It is planned to develop further products with other EPN species and to expand the functionality of the capsule.

Selective breeding of biocontrol agents; lessons learned from the BINGO project

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In horticulture, the crops that are grown are the result of long-running and highly advanced breeding programs. These programs have been very successful and have a very large impact throughout. In contrast, for the biological control agents that are applied to protect these crops from pests, selective breeding has hardly been used at all. From 2015 to 2018 the BINGO-ITN (Breeding Invertebrates for Next Generation BioControl) project was executed. BINGO-ITN hosted 13 Early Stage Researchers (ESRs) that each worked on a project related to the breeding of biocontrol agents. The project was successful in training the ESRs and establishing an international network. It also successfully generated genomic data for some important biocontrol agents and much many valuable insights. However, generating new varieties by selective breeding turned out to be very hard. It requires establishing genetically diverse populations to start the selection process, phenotyping for complex characters on very small and short- lived animals, and the maintenance of many different populations and selection lines in parallel. In this presentation we will discuss why each of these requirements are challenging, and how these challenges may be resolved in the future.

Results of the application of microorganisms in maize and maize-bean intercrops to promote plant health

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Intercropping of maize (*Zea mays* L.) with bean (*Phaseolus vulgaris* L.) or other crops has gained a considerable amount of attractiveness in Germany over the last years. Originally used in South America in a slightly modified form, this cropping system can make a significant contribution to the reduction of pesticide applications, the enhancement of biodiversity, soil fertility and plant as well as soil health. Plant-growth promoting microorganisms (PgPMOs, e.g. mycorrhizal fungi and their metabolic products) interacting with the plant rhizosphere can positively influence overall health and stress tolerance of crop plants. From 2018 to 2021, the effect of such PgPMOs in maize-bean intercrops was studied in a series of field and greenhouse trials at four locations in Baden-Württemberg, Germany (Rheinstetten-Forchheim and Wendelsheim: exact trials, Stetten a.H.: practical field trials, Karlsruhe: greenhouse trials) (Dieckhoff et al. 2022). In these trials, commercial Mycorrhiza and *Trichoderma* products were used in open field trials as well as in rhizotrones under controlled conditions in the greenhouse. In the exact trials, the products Agtiv® (powder formulation, mycorrhiza plus bacteria, Premier Tech Agriculture) and Avengelus® (granulate, *Trichoderma atrobrunneum* T-720, MycoSolutions) were used, in the practical field trials the product Xilon® (granulate, *Trichoderma asperellum* T34, Kwizda Agro), and in the rhizotrones the products Agtiv®, Avengelus® (granulate and liquid formulation) and Xilon®. In the rhizotrone tests, an increased root development and a higher root colonization by the PgPMOs contained in Agtiv® and the two Avengelus® formulations were observed. In the exact trials, variability in the root colonization capacities was observed depending on the year and the location. None of the products, however, led to significant differences in yields or improved nutrient cycling in maize single crops or maize-bean intercrops. In addition, the formulation of the products in combination with the seed drill technology had an impact on the outcome of the inoculation as measured by the degree of root colonization and the germination of seeds. While the Agtiv® components blended well with the individual seeds und were easily spread with customary drill technology, the Avengelus® granulate could only be deployed successfully via an underfoot fertilizer or a fertilizer attachment during the sowing process; otherwise, the seeds were unevenly distributed during the precision spaced planting process. In the practical field trial, an improved plant height as well as increased root mass of the maize plants were observed. The results of these studies as well as the positive findings of another trial testing mycorrhizal products in cultivated blueberries where an increase in new shoots, root mass, and longevity was observed (Zunker et al. 2017), encourage further field trials with selected PgPMOs under different cultivation conditions.

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ZUNKER, M., REIßIG, A., & SCHNELLER, H. 2017. Mykorrhiza-Bodenhilfsstoffe in Kulturheidelbeeren. Erste Ergebnisse der Erprobung von zwei ericoiden Mykorrhiza-Isolaten an Kulturheidelbeeren *Vaccinium corymbosum* (Amerikanische Heidelbeere) am Landwirtschaftlichen Technologiezentrum Augustenberg (LTZ). Deutsche Baumschule, 11.

The dangerous life of the lime-tree aphid *Eucallipterus tiliae*
(mit englischen Untertiteln)

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Der Videofilm (Dauer 15 min.) beginnt mit einer kurzen Vorstellung der Lebensweise der Lindenzierlaus *Eucallipterus tiliae* auf einer Winterlinde (*Tilia cordata*) und zeigt u.a. wie Nymphen und geflügelte Läuse überschüssigen, aus Siebröhren aufgenommenen zuckerhaltigen Saft in Form von Honigtautropfen weit wegspicken. Danach richtet sich der Fokus auf die natürlichen Feinde, zuerst auf die Mumien verschiedener Parasitoide und dann auf die Prädatoren: Gezeigt wird der Beuterewerb juveniler Blumenwanzen (Beispiel *Anthocoris nemorum*) und Weichwanzen (Beispiel *Deraeocoris lutescens*) sowie von frisch geschlüpften Florfliegenlarven. Besonders bemerkenswert ist der Beutefang von Schwebfliegen-Larven: Es wird gezeigt, wie eine juvenile Blattlaus bei zufälligem Kontakt mit einer frisch geschlüpften Schwebfliegen-Larve an dieser irreversibel kleben bleibt und schließlich mit dem Mundhaken ausgeschabt wird bis nur noch die Hülle übrigbleibt. Selbst geflügelte Blattläuse erleiden dasselbe Schicksal, wenn sie mit dem Fühler an einer größeren Larve kleben bleiben und sich nicht mehr loslösen können. Hungrige Maienkäfer verschlingen Blattläuse restlos, sogar samt Flügel, Beispiel *Harmonia axyridis*. Lehrbuchhaft ist eine besondere Sequenz, welche die Wirkung des Alarmpheromons einer angegriffenen Blattlaus auf die Fluchtreaktion benachbarter Blattläuse veranschaulicht.

New knowledge on the brown marmorated stink bug and its natural enemy *Trissolcus japonicus*

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The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) is native to Asia and arrived in Europe in the early 2000s. Since its arrival it has spread through most of Europe's mainland, and due to climate change it has the potential to further expand its range. Because its polyphagous behaviour, *H. halys* quickly became an invasive pest of a wide variety of tree fruit, nut, vegetable, and field crops in Europe, particularly in Italy and Georgia, where it has caused severe economic losses in tree fruit and nuts. As the impact of native natural enemies on invasive *H. halys* populations in Europe is generally low, *H. halys* was identified as promising target for classical biological control in the invaded range. Surveys for natural enemies in Asia revealed that it is mostly attacked by egg parasitoids, among which the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), was identified as the most promising candidate for classical biological control. Prior to the release of exotic biological control agents into a new environment, it is important to consider the potential for unintended non-target effects such as host-range expansion to native or beneficial species. Accordingly, host specificity testing of *T. japonicus* has been carried out under containment conditions in Europe. Developmental suitability of non-target host species for *T. japonicus* was demonstrated in no-choice tests by successful offspring emergence from 11 of 13 non-target species tested. Whereas most non-target species were less accepted than *H. halys*, four native species [*Palomena prasina* (L.), *Rhaphigaster nebulosa* (Poda), *Pentatoma rufipes* (L.), and *Arma custos* (F.)] were accepted at rates comparable to the target host. As laboratory risk assessment studies for *T. japonicus* in Europe were underway, adventive populations of *T. japonicus* were discovered in Switzerland, Italy, and Germany. Following the unintentional introduction, the ecological host range of *T. japonicus* in the invaded range in Switzerland was investigated by exposing sentinel egg masses of *H. halys* and native non-target species. Egg masses of *P. prasina* and *P. rufipes* exposed on trees were regularly parasitized by *T. japonicus*, whereas species feeding on weeds were not attacked at all. Since *P. rufipes* starts laying eggs in late August when *T. japonicus* populations are highest, it was the most frequently parasitized non-target species. Ongoing life table studies in Europe may help to understand if the arrival of *T. japonicus* has any negative consequences for *P. rufipes* populations.

Application of pupal parasitoids for the biological control of *Drosophila suzukii* (Diptera: Drosophilidae) in berry fruit production – ParaDrosu

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The invasive spotted wing drosophila (*Drosophila suzukii*) has become a main pest in protected berry fruit production in Europe. This is due to the development of several generations per year and the capability of females to infest undamaged soft-skinned fruits of various wild and cultivated host plants. Insecticide applications are very common but often inefficient due to the infestation of ripening or ripe fruits and long harvest periods while having negative effects through insecticide residues when applied close to harvest. Exclusion netting works well, but is cost intensive and sometimes not sufficient to control emerging populations. Luckily, the native pupal parasitoids *Trichopria drosophilae* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae) have been found to successfully parasitize *D. suzukii* and thus have a great potential as biological control agent that may supplement current measures. The research project "ParaDrosu" (2021-2024) aims for developing an innovative biological management strategy based on the release of these pupal parasitoids. Aspects investigated include successful mass rearing of parasitoids which requires suitable hosts, the right climatic conditions and diet for the adults. Moreover, the rearing system has to be efficient as well as economically viable. In this context, parasitoid populations from different areas in Germany are collected and tested for their performance with the aim of being introduced into the existing rearing strains to maintain parasitoid quality. Performance under different climatic conditions is tested and optimal storage conditions are defined. An appropriate parasitoid application technique ensuring both optimal protection during transport and release and sufficient dispersal in the crop will be developed. Furthermore, the best release times, intervals and parasitoid numbers will be determined. Relevant plant protection products are tested for negative side effects on the parasitoids to define possible incompatibilities as well as suitable timing for integrated pest management strategies. Semi-field and field trials are conducted in Southern Germany to examine the parasitoids' efficiency in controlling *D. suzukii* in berry fruit cultivation. Results from the described research project should lead to defining a biological control agent for mass production as well as detailed instructions to successfully control *D. suzukii* in protected berry fruit cultivation under Middle European climatic conditions.

The project "ParaDrosu" – Biological Control of *Drosophila suzukii* by application of native pupal parasitoids – a closer look

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Drosophila suzukii Matsumura (Diptera: Drosophilidae) is an invasive pest of fruit and berries. The attack of undamaged ripening fruit results in challenges for pest control, and effective means of biological control to regulate D. suzukii in Germany do not exist yet. In its native range, *D. suzukii* is attacked by different hymenoptera, such as the cosmopolitan pupal parasitoids *Trichopria drosophilae* Perkins (Diapriidae) and *Pachycrepoideus vindemmiae* Rondani (Pteromalidae). European populations of both species have shown to successfully parasitize *D. suzukii*. Prior to application of parasitoids on a commercial scale, a suitable application rate has to be determined and specified for different types of cultures. Furthermore, the “area of activity” is of interest, i.e. whether a parasitoid is active near ground level or in the berry zone. To answer these questions with a focus on protected berry cultures, a model trial was started on the experimental field of Julius Kühn-Institute in Dossenheim. Three raspberry plantings (each sized 20 m²) were netted with a standard net (mesh 0.8 cm); one additional planting was free-standing. All plantings were artificially infested with *D. suzukii*. One netted planting each was subject to weekly releases of five female *T. drosophilae*/m² or five *P. vindemmiae*/m²; the third netting and the free-standing planting served as controls. To determine parasitism activity, raspberries were baited with fresh *D. suzukii*-pupae and weekly exposed in the plantings for five days from August to mid October. Afterwards, the pupae were extracted and incubated. Of the 6400 exposed pupae, 4272 were retrieved in the laboratory, from which 3435 insects eclosed (2508 *D. suzukii*, 401 *T. drosophilae*, 526 *P. vindemmiae*). The maximum parasitism rate in a planting was 39.8 % for T. drosophilae and 45.4 % for *P. vindemmiae*. The latter was mostly detected in the upper berry zone (120-190 cm) and most active from mid September to October, while *T. drososphilae* was mostly detected near ground level and did not have a temporal focus. Both species parasitized bait pupae in all four plantings. The first model trial in summer 2021 demonstrated a suitable method for quantifying parasitoid activity, but showed the limitations of testing while using a standard net to separate different treatments. Further model trials in 2022 will focus on comparing the efficacy of the two species under modified testing conditions, such as a lower release rate, and will hopefully help to better understand the activity of released parasitoids in protected berry cultures.

The project Antago-Senecio: herbivorous insects for the control of tansy ragwort

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The rising number of tansy ragwort (*Jacobaea vulgaris* Gaertn.) in grassland, especially under low management intensity, is highly problematic for grazing livestock due to the poisonous impact of pyrrolizidine alkaloids in these plants. This phenomenon also exacerbates the use of those ragwort-rich grasslands for grazing or hay and silage production. Current management and control practices are time consuming or rely on herbicides which may be banned in future, wherefore an innovative and self-regulating method is in need to secure the livelihood of farmers and the preservation of diverse and protected grasslands. Therefore, one aim of the EIP-Agri-Project "Antago-Senecio" is finding local and specialized herbivorous insects as potential biological control agents for the regulation of tansy ragwort. Here, the well-known cinnabar moth, *Tyria jacobaeae* L. (Lepidoptera, Arctiidae) and ragwort flea beetle, *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae), as well as the less studied ragwort crown boring moth, *Cochylis atricapitana* Stephens (Lepidoptera: Cochylidae) and the ragwort fly, *Sphenella marginata* Fall. (Diptera: Tephritidae) may be an option.
First collection of these insects was performed in the season 2022 and more than 150 larvae of *T. jacobaeae* were collected from several locations. However, at least two populations were infected by microsporidia and only 26 % of *Tyria*-larvaes survived and pupated successfully. The mean weight of the 44 surviving *Tyria*-pupae is 142.59 ± 21.81 mg, representing a solid weight for further development. Additionally, *L. jacobaeae, S. marginata* and *C. atricapitana* were collected in the field and a rearing in the greenhouse could be started. Future laboratory tests will aim on the establishment of successful rearing technique as well as feeding trials to evaluate the harmful effects of the antagonists. First successes resulted in rearing *C. atricapitana* on larval medium and the establishment of a 3rd and 4th generation in the greenhouse.
Besides the suitability of the antagonists regarding the control of *J. vulgaris*, their effect on other ragwort species like *Jacobaea aquatica* G. Gaertn., B. Mey. & Scherb, *Jacobaea erucifolia* G. Gaertn., B. Mey. & Scherb and *Senecio inaequidens* D.C. will also be investigated. Moreover, field tests in the Westerwald in Hesse and Rhineland-Palatinate will be conducted and will complement the laboratory studies. Finally, the result will be used as basis to evaluate the potential of these herbivorous insects to regulate and/or prevent the dominance of ragwort in low-intensively used grasslands as well as perspectives of their long-term establishment and release strategy in grasslands.

Acknowledgments: The agricultural European Innovation Partnership (EIP-Agri) project "Antago-Senecio" (No. 71588337) is funded through the European Agricultural Fund for Rural Development (EAFRD), co-financed by Hesse and is implemented by the Hessian program for rural development (EPLR 2014 – 2020). Project partners are the Justus-Liebig-University Giessen (project coordination) and eight farmers from Hesse and Rhineland-Palatinate.

ResBerry – Resilient organic berry cropping systems through enhanced biodiversity and innovative management strategies

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Monoculture does not represent the ecological optimum due to the loss of various ecosystem services. Consumer awareness of near-natural cultivation methods is increasing, resulting in a significantly higher demand for organically produced food. This trend can also be seen in berry production. Since organically produced berries are often negatively affected by various pests and diseases as well as new invasive pests, there is an urgent need for robust and resilient cultivation systems. ResBerry is a newly launched EU-project funded under Core Organic Cofund. The main objective of the project is to increase resilience of European organic berry orachrds against major pests and diseases through enhanced above- and belowground biodiversity. Accordingly, ResBerry will focus on: a) Implementation of preventive pest control measures through identifiying suitable habitats and its management for natural enemies in organic berry orchards by including companion plants. For example, dispersal of selected target pest insects and their antagonist from companion to crop plants and vice versa will be assessed; b) Deciphering the soil microbial community in organic berry orchards, how communities are shaped by companion plants, and which measurements can be applied to favour beneficial soil microorganisms as a preventive strategy against soil-borne pathogens and for overall increased resilience; c) Raising awareness among farmers for using direct innovative, but so far little implemented curative pest control strategies, such as entomovectoring for control of grey mould; d) Evaluating the implications of the proposed measures on yield and nutritional quality of berries and tackling the consumers' expectations, acceptance and preferences regarding the newly developed production systems and their produced fruits; e) Disseminating and communicating the results to stakeholders, growers, market organizations, research scientists, academia, technical services and consumers. With a wide geographical coverage in five European countries, the project will focus on strawberries and raspberries but will also consider other small fruits as well. This multidisciplinary approach delivers systemic solutions supporting organic farming systems to reduce the dependency on external inputs as fertilizers and plant protection products, while still increasing economic sustainability and restoring biodiversity.

“Solar Beneficial Insects”: promoting beneficial arthropods in agrophotovoltaic systems

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In order to achieve climate protection targets set by the German Government, an enormous expansion of renewable energy will be necessary, including photovoltaic (PV) systems. To avoid a land-use conflict between agriculture and energy production, agrophotovoltaic (APV) systems could be increasingly used. In APV systems, the PV modules are either installed elevated above the crop itself or vertically next to the crop, so that the area below or in between the modules can still be used agriculturally.

Currently, there is still a substantial knowledge gap regarding the compatibility of the dual agricultural and PV use. Especially, the potential impact on the functional biodiversity as an important guarantor for healthy crops is still unexplored in APV systems. Therefore, the aim of this research project is to determine how APV systems can be designed to support settlement and promotion of beneficial arthropods and hence reduce certain pests.

The project has started recording and evaluating current activities regarding APV systems in Germany, especially with respect to functional biodiversity. In cooperation with operators of APV systems the diversity and abundance of beneficial arthropods around existing APV systems, with a focus on hoverflies, Spheciformes wasps and spiders will be investigated. In order to promote beneficial insects the project will also develop and field-test the performance of various elements that can be integrated in APV systems (e.g. flowering strips or nesting aids installed in the mounting fixtures). Additionally, the project aims to investigate whether the PV modules themselves have a direct influence on specific beneficial insects and pests, e.g. a repelling or attracting effect.

Overall, the project aims to provide knowledge about how APV systems can be designed in an environmentally friendly way generating additional benefit to agriculture through pest control and pollination.

„nützLINK“ – a Citizen Science based approach to monitor beneficial arthropods in agricultural landscapes in Germany

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The global loss of biodiversity and in particular the decline of insects has serious consequences for many ecosystems worldwide. In agriculture, important ecosystem services such as crop pollination and natural pest control are at risk. Due to agricultural intensification, beneficial arthropods such as predators and parasitoids are frequently exposed to pesticides and fertilizers. Lack of (semi-)natural structures prevents them from recovering in habitats less polluted by chemical inputs. Regular monitoring of their populations in agricultural landscapes is therefore necessary to detect changes in their communities and disturbances of their ecosystem services. On the other hand, a standardized monitoring program may also help to record positive effects of agroecological measures on biodiversity. Adult hoverflies are important pollinators, while the larvae of many species are effective predators of various field crop pests (e.g. aphids). Establishing a long-term monitoring of their populations will serve as an indicator of the condition of agricultural landscapes as part of the collaborative project MonViA# (National Monitoring of Biodiversity in Agricultural Landscapes). Due to their life cycle and high mobility, many hoverfly species roam in very different habitats. Their populations are influenced by the availability of resources and potential disturbance factors across a wide spatial range. Orchard meadows embedded in the agricultural landscape are extensively managed agroforestry systems that provide temporary refugia and resources for many insect groups, including hoverflies. As low-disturbance ecotones, they can serve as a spatial matrix for establishing a long-term monitoring of the target insect taxa. Furthermore, many orchard meadows are maintained by citizens, communities and associations. This network of interested stakeholders can contribute to document the occurrence of hoverflies and other beneficial insects within the Citizen Science approach "nützLINK" of our project (https://www.agrarmonitoring-monvia.de/en/news-details/nuetzlink-goes-online). The recording of parameters such as biomass, abundance and diversity of the target taxa, and the assessment of their population trends is currently performed with a combination of traditional (yellow pan traps, malaise traps) and new methods where Citizen Scientist can be involved. These methods include the use of eDNA analyses##, the development of artificial flowers and a camera trap for automated monitoring of flower-visiting insects. The concept of this monitoring program and initial results are presented in this paper.

#On behalf of the Federal Ministry of Food and Agriculture, a total of 12 specialist institutes of the Thuenen Institute and the Julius Kühn Institute as well as the Federal Office for Agriculture and Food are working together in the project MonViA (https://www.agrarmonitoring-monvia.de/en/).
##in cooperation with Sinsoma GmbH, Austria

The following abstracts were presented during the 37th Annual Meeting of the Working Group "Beneficial Arthropods and Entomopathogenic Nematodes". This meeting was held from 26. To 27. November 2019 at LTZ Augustenberg in Karlsruhe and was perfectly organized by Dr. Mareille Zunker from LTZ.

Natural enemies of invasive pest insects in Southwestern Germany – unrecognized, but already efficient

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Effective natural enemies are often missing against introduced invasive insects. In the invaded region generalist antagonists, cold winter conditions and chemical control cannot reduce invasive species. Introducing specific parasitoid antagonists to increase the mortality to a usual level seems to be a necessity to solve the problem. It is necessary to investigate the potential of suitable species to avoid failures like a low effectivity or even non-target effects. There are no examples known in Europe with a negative environmental impact. The misunderstood case of the established Asian ladybird beetle *Harmonia axyridis*, a generalist predator, was no classical biocontrol against invasive pests. It is an example for the utilization of a beneficial insect without prior estimation of environmental risks.
Controlling the San-José scale from Asia by establishing a parasitoid from America saved orchards in Southwestern Germany in the 1960ies. The scale pest was already resistant to chemical control. This is a successful example for classical biological control. Today there are often concerns against this practise although candidate species are being selected carefully. Several unrecognized positive examples show that invasive insects are already being controlled by introduced parasitic hymenoptera in Southwestern Germany. They have been released in neighboring countries and then spread naturally to Gemany.
The White cicada *Metcalfa pruinosa* is being parasitized by *Neodryinus typhlocybae* in many locations in the Rhine valley. A parasitism rate of about 10% is still rising. To control the Chestnut gall wasp *Dryocosmus kuriphilus* two *Torymus* species had been released in Italy. Only five years after the first observation of this former quarantine pest the parasitism rates reached 70-100% also in Southwestern Germany. More examples include natural enemies of several scale insects and the Buffalo treehopper. They had never been released in Germany but can be found as effective antagonists of these invasive pests.
Against invasive bugs like the Brown marmorated stink bug *Halyomorpha halys* there are no effective antagonists in Europe. *Trissolcus japonicus* was found to have been accidentaly transported to Italy and Switzerland and could be part of a control strategy. #
Although it is proven that classical biological control can be effective, the legislation for this plant protection method has not been harmonized in Europe. Unfortunately this preventative plant protection method is not being supported as it should be although the economic losses will cost several Mio € even in a single species like *H. halys*. Chemical plant protection alone cannot control invasive insect species.

#: Comment by editor: In the meantime the wasp has found in Germany as well:
Dieckhoff, C. et al. (2021): Add Germany to the List-Adventive Population of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) Emerges in Germany. Insects 12(5):414. doi: 10.3390/insects12050414.

The project ProgRAMM: monitoring and mapping of invasive insects

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The german project ProgRAMM has the objective to update the pest risk analysis of climate sensitive insects in agricultural crops. The project is a cooperation between the Julius Kuehn-Institute (JKI), the Potsdam Institute for Climate Impact Research (PIK) and the Agricultural research Center (LTZ).
The LTZ is providing biological data of six invasive insect species with different distribution strategies. Southern green stink bug *Nezara viridula*, Brown marmorated stink bug *Halyomorpha halys*, Mediterranean fruit fly *Ceratitis capitata*, Cotton bollworm *Helicoverpa armigera*, Obscure mealybug *Pseudococcus viburni* and European pear scale *Epidiaspis leperii*.
The recently most popular invasive species is *H. halys*. It has been recorded in Germany since 2011 and has spread along the upper Rhine valley and via traffic to several larger cities. *N. viridula* has been recorded since 1979. It benefits by climate change due to moderate winter conditions. The two species *C. capitata* and *H. armigera* are occurring regulary in Germany. *C. capitata* causes local damage in e. g. peaches and the larva of *H. armigera* attacks tobacco and corn. Yet it is unknown if these two species are able to survive overwintering conditions in Germany. *E. leperii* and *P. viburni* are the two immobile species in the project. *E. leperii* has been recorded since 150 years and is spreading in the last years. In Germany *P. virburni* occurs usually in greenhouses on ornamentals, but they also been a field record in German urban area on Catalpa. This was the proof for the ability to overwinter in central Europe.
Methods for monitoring are specific for each of the six insect species. Principally visual monitoring is the key method. Trapping is possible for only *H. halys*, *C. capitata* and *H. armigera. E. leperii* has to be detected by scratching the branches surface of e.g. of pear and plum since this scale insect is hiding under green algae covering. *N. viridula* can only be observed visually. Monitoring the distribution of the obscure mealybug is a challenge.
The data collection is supported by ISIP (www.isip.de), using the internet application ArcGIS-Collector. The distribution maps are being display in realtime on any internet-website using the specific embed codes. Further information on the project ProgRAMM can be found here:
https://ltz.landwirtschaft-bw.de/pb/,Lde/Startseite/Arbeitsfelder/ProgRAMM
Contributions of record data of these invasive insects to our data base are welcome.

This project is being funded by the German Federal Agency for Agriculture and Food
(pt ble), FKZ 281B204716.

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. However, other than in vineyards or orchards, 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. Therefore, 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 winter and in May during pruning of vineyards, cut them into small pieces and dispersed them in experimental hop gardens. The cuttings in May have been brought into the hop garden immediately, those from winter in the same week in May. We also used purchasable predatory mites in form of a mix of *Phytoseiulus persimilis* and *Neoseiulus californicus* on bean leaves as this was the most successful way of dispersal in the first year of the project.
In 2019 infestation by *T. urticae* was less severe than in 2018 why results from only one of the five experimental hop gardens yielded significant differences. Interestingly, lower yields of some variants were balanced by higher alpha acid contents and therefore no significant difference in amount of alpha acids per hectare was found. Besides, we demonstrated that lower yield and alpha acid content in another field had not been caused by spider mite infestation but by differences in soil quality.

Heterorhabditis downesi, a new nematode for horticulture

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For the first time in 15 years E-Nema introduces a new entomopathogenic nematodes species, *Heterorhabditis downesi*, into the market. *Heterorhabditis downesi* was known as the Irish strain of *Heterorhabditis megidis*, until it became an own species in 2002. *Heterorhabditis megidis*, a nematode widespread in Europe, has been applied for vine weevil control until cessation of its production in 2012. Heterorhabditis downesi has been found in Ireland, Scotland, England, Denmark, Italy and Hungary. It has a preference for sandy soils in the transect from the front of the dunes to the grassland. In October 2019 researchers of E-Nema isolated it from the sandy coastal zone of the Baltic Sea in Germany. E-Nema's main reason to market a new nematode is the gap in the vine weevil control at low temperature in early spring and late autumn. Vine weevil larvae start feeding at 5 °C, but *Heterorhabditis bacteriophora*, E-Nema's standard nematode for its control, infects only at 12°C. *Heterorhabditis downesi* infects insects and causes host death at temperatures between 8° and 35°C and can be applied both at low and high temperatures. Other characteristics of this new nematodes are a good persistence in storage and in the field and a broad host range, which comprises vine and pine weevil, chafer grubs and cutworms. *Heterorhabditis downesi* is particularly effective against other *Otiorhynchus* species such as *Otiorhynchus armadillo*, *Otiorhynchus salicicola* or *Otiorhynchus dieckmanni*. Field trials in Ireland show good performance against vine weevil larvae in strawberries in spring and autumn application. Field trials in Germany are presented in the following presentation.

Efficacy of entomopathogenic nematodes *Heterorhabditis downesi* and *Steinernema kraussei* against the larvae of the black wine weevil (*Otiorhynchus sulcatus*) at low temperatures.

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When entomopathogenic nematodes are used in horticultural practice against larvae of *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), low temperatures often lead to unsatisfactory efficiencies. Therefore *Heterorhabditis downesi* and *Steinernema kraussei* with 0.5 million/m² in 0.5 l pots with yews (*Taxus baccata*) at 15°C day and 8°C night temperatures (12h each) were tested in a climatic cabinet (n = 24). Of four L6 larvae previously placed in the substrate, an average of 3.1 were still alive in the control treatment four weeks after application, compared to 0.1 in the treatment with *H. downesi* and 0.7 with *S. kraussei*.
In a second experiment (n = 20), day temperatures were 12°C and night temperatures were 6°C (12h each). Initially *O. sulcatus* eggs were placed on the soil and on average 4 larvae (L2 to L6) developed from 2 x 10 eggs. Application of *H. downesi* reduced the number of larvae to 1.7 and *S. kraussei* to 1.2 live black wine weevil larvae. With a 50% reduction in the application rate for *H. downesi* to 0.25 million/m², the same efficacy was achieved as with 0.5 million/m². The combination of *H. downesi* with *Steinernema feltiae* (1:1, 0.5 Mio/m²) did not show a synergistic effect.

Monitoring of some pest insects by entomopathogenic nematodes in Georgia

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The goal of the present study was to determine the efficacy of EPNs of five entomopathogenic nematode species (*Steinernema carpocapsae, Steinernema feltiae, Heterorhabditis bacteriophora, Heterorhabditis poinari* Kakulia, Mikaia, *Bursaphelenchus* sp.; 200,300IJs/ml. concentration)) have been used against various important insect pests like Leaf Curl Plum Aphid, (*Brachycaudus helichrysi*), citrus mealy bug (*Planococcus citri*),melon aphid (*Aphis gossypii)*, fern scale (*Pinnaspis aspidistrae*), Black vine weevil (*Otiorhynchus sulcatus*) and *Halyomorpha halys* known, as Brown Marmorated Stink Bug (BMSB), which today is very dangerous pest in Georgia. All pest insects were obtained from infested plants. Laboratory experiments were conducted at temperature 25º C and RH 72%. Treatments of nematodes (300 IJs) on larvae and imagines of the different pest insects were evaluated on the 7th day after treatment. From nematode strain *S. carpocapsae* from e-nema company, Germany on all pest insects we received 82.4, 84.3, 78.4, 85.4, 77.4, 84.6, 76.4, 85.6, 62.4, 72.3, 77.7, 86.1 % mortality. From *S. feltiae* on all pest insects we received 68.5,79.6, 73.5, 78.6, 63.4, 68.2, 71.4, 72.6, 58.5, 60.6, 74.4,76.6% mortality.

From nematode strain *S.carpocapsae*, origin Israel, on all pest insects we received: 70.2,72.4, 74.2,76.4, 76.2,78.4, 74.2, 77.5, 59.2, 62.3, 75.2, 76.5% mortality. From *S. feltiae*, on all pest insects we received: 64.5,70.6, 65.6, 75.4, 64.6, 74.66.4, 75.4, 48.5, 50.6, 68.4, 76.7% mortality.

From nematode strain *H. poinari* Kakulia et Mikaia on all pest insects we received: 56.4, 61.4, 58.4, 62.4, 58.5, 64.2, 58.6, 65.0, 39.4, 41.4, 56.8, 64.0% mortality.

Laboratory bioassays also were conducted to determine the effectivity in the combination of entomopathogenic nematodes (300 IJs/ml) and entomopathogenic fungi (*Beauveria bassiana;* 2.5 X 105 conidia per larva) to control *H. halys*. From German strains (e-nema company) *S. carpocapsae*, *S. feltiae* and *H. bacteriophora*, we received highest result of mortality 90.2, 95.5, 84.2, 88.0, 78.5, 81.4% on *H. halys*. Strains with origin from Israel *(S. carpocapsae, S. feltiae, H. bacteriophora*) achieved high mortality (82.4, 82.3, 80.2, 81.0, 74.8, 78.4%) on *H. halys*. Strains from Georgia (*Bursaphelenchus* sp. and *H. poinari* Kakulia et Mikaia) mortality rates beween 54.5 and 68.7 were observed on *H. halys*.

We received high mortalities of all tested pest insects by treating them with five different nematode species in laboratory testing. Further studies considering green-house or field experiments are warranted to expand on this knowledge.

Methods of monitoring hoverflies (Diptera: Syrphidae) and their fitness conditions in agricultural landscapes in the FInAL-project

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In times of decreasing natural habitats, agricultural landscapes become more and more important in supporting populations of insects. They are taking up to 50 % of the land area in Germany and so they have a great impact on the richness of species and the steady declining of insect abundances. Many insects provide important services including pollination, pest control or being a food source for other animals like birds or amphibians.

Adult hoverflies are important pollinators for example of strawberries or fruit trees. Females need pollen for oogenesis and nectar plays a major role for growth and development of adult individuals. 39 % of the larvae are zoophagous and therefore play a major role as biological pest control agents against aphids. The aim of FInAL (facilitating insects in agricultural landscapes) is to introduce renewable resources into existing cultivation systems which can support local insect populations by providing essential resources, are economically workable and acceptable for farmers. An important role plays the implementing of integrated pest management.

Syrphidae are significant for this project because they are responsible for pollination and pest control. In order to provide these ecological services, it is necessary to research foraging of hoverflies, the abundance and species diversity and how they can be supported by sustainable cultivation systems. The first step is to study the abundance of hoverfly species in the field laboratories before and after planting renewable resources and if this measure is going to have an effect. Therefore, hoverflies are going to be trapped with yellow, blue and white pan and Malaise traps and identified to species level. The second step is to explore several fitness parameters of the hoverfly population in the altered landscape. One factor that we want to study is the use of flower resources provided by the new cultivars by hoverflies and potential effects on fertility and energy resources. Gut analyzes provide information about the acceptance of the new food source and which role it may play in foraging of hoverflies. Pollen analyses will be performed to make statements about how important the cultivars are in proportion to the entire food of hoverflies and how they are going to be accepted as alternative resource. In preliminary tests we are trying to extract the pollen from hoverflies via dissection of the abdomen. This is also possible through examination of fecal pellets, which give us the option of researching the captured individuals alive. Additional analyzes of nutrient, fructose, glucose and lipid levels will give us valuable information about the energy that is available for the hoverflies and which plant is more suitable to support insect populations.