turned out that the QuEChERS-method is an efficient way to extract dtxs from different crop matrices. Using offline SPE for the clean-up of the samples analytes can be separated from disturbing matrix compounds and quantified by the UHPLC-DAD/TOF-MS method.

Contributed paper. Monday, 17:45. **57-STU**

α-1, 2-mannosyltransferase ktr1, ktr4 and kre2 regulate positively growth, conidiation, viability, virulence, and multi-stress tolerances in *Beauveria bassiana*

Juan-juan Wang, Lei Qiu, Sheng-Hua Ying, Ming-Guang Feng*
Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China
Address for Correspondence: mgfeng@zju.edu.cn
E-mail address of the presenting author: wjj880414@163.com

Entomopathogenic fungus *Beauveria bassiana* is a mycoinsecticide against arthropod pests. Three α-1, 2-mannosyltransferase proteins (Ktrp) named Bbktr1, Bbktr4 and Bbkre2 are responsible for extension of the second and third mannose residues on secretory protein. Here, we characterized the role of three Ktrp in *B. bassiana* and found that they were positive, but differential, regulators of the growth, conidiation, multi-stress tolerance and virulence of the entomopathogenic fungus. The three disruptions accompanied with their corresponding complement Δ*Bbktr1/ktr1*, Δ*Bbktr4/ktr4*, Δ*Bbkre2/kre2* and wild-type were constructed. Δ*Bbktr4 and* Δ*Bbkre2* grew 50–83% slower on nutrition-rich and limited media while Δ*Bbktr1* show similar colony sizes on all the tested media. Their conidial yields on a standard medium were reduced by 31–96%, accompanied with abnormal germination. All the mutants became significantly less tolerant to most stresses of cell wall perturbation, high osmolarity, oxidation, wet heat and UV-B irradiation during colony growth. Furthuremore, the Ktrp mutants were altered in cell wall structure and composition, which contributed to the thickness of cell wall, increased sensitivity to lyase, the low conidial hydrophobicity and cell surface carbohydrate epitopes. Coincidentally, the attenuated cell wall in Ktrp mutants also brought out the more protoplast to release. Remarkably, insect bioassays revealed decreased virulence in Δ*Bbktr4*, Δ*Bbkre2* for 18% and 1.2-fold with topical application, and 31% and 26% with intrahemoceol injection. Our findings revealed that Ktrp plays a central regulatory role in *B. bassiana*.

TUESDAY - 5 August

SYMPOSIUM 3 (Fungi) Tuesday, 8:00-10:00

Fatal attraction: Fungi and Odours in Deadly Combinations for Pest Control

Symposium. Tuesday, 8:00. **58**

Conifer - bark beetle - fungus interactions

Tao Zhao[1], Paal Krokene[2], Anna-Karin Borg-Karlson[1]
[1]The Royal Institute of Technology, Department of Chemistry, Ecological Chemistry Group, Stockholm, Sweden; [2]Norweigian Forest and Landscape Institute, Ås, Norway
Address for Correspondence: taozhao@kth.se

Tree-killing bark beetles such as the spruce bark beetle (*Ips typographus*) have huge economic and ecological impacts in conifer forests worldwide. Just in the last 25 years the spruce bark beetle has killed millions of cubic meters of Norway spruce (*Picea abies*) in Europe. Trees are killed by a combination of pheromone-mediated mass-attacks and infection with phytopathogenic bluestain fungi vectored by the beetles. *Ceratocystis polonica,* the most virulent fungal associate of the spruce bark beetle, can kill healthy trees in the absence of beetle attack if it is experimentally inoculated into the bark at high densities. Norway spruce protects itself against combined beetle-fungus attacks by multiple preformed and inducible defense mechanisms. Structurally diverse mixtures of mono-, sesqui- and diterpenes are central components of these defenses. Preformed terpenes stored in resin ducts in the bark and sapwood may repel or inhibit initial attacks. Terpene levels increase tremendously following induction by e.g. fungal infection or application of methyl jasmonate (a defense-inducing plant hormone). This induced terpene response reduces pheromone emission by the spruce bark beetle and inhibits tree colonization in a dose-dependent manner. However, fungal associates of the spruce bark beetle can greatly reduce monoterpene levels in the tree by biotransforming them to oxygenated monoterpenes. In addition, the fungi also produce different metabolites which may play multiple roles in bark beetle host finding and colonization. These observations demonstrate the complicated interactions between conifer-bark beetle-fungi.

Symposium. Tuesday, 8:20. **59**

Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi

Mario Schumann[1]; Anant Patel[2]; Miriam Hanitzsch[2]; Stefan Vidal[1]
[1]Georg-August-Universität Göttingen, Department of Crop Sciences, Göttingen, Germany; [2]Fachhochschule Bielefeld, University of Applied Sciences, Department of Engineering and Mathematics, Bielefeld, Germany
Address for Correspondence: svidal@gwdg.de

The larvae of soil dwelling insects use carbon dioxide gradients, established by growing roots, to orientate towards their host plants. This long distance orientation cue is complemented by other volatile cues to finally accept a host plant for feeding. Previous application strategies using entomopathogenic fungi for soil pest control were using high concentrations of spores per m^2, set against competing microorganisms in the rhizosphere. In the attract and kill approach the strategy is turned upside down: larvae voluntarily make their way to the spores, contained in capsules emitting CO_2. When near to these capsules, probability of larval infestation with spores is higher. However, to make this strategy work, the capsules need to fulfill several prerequisites, such as building up a gradient significantly higher than the background CO_2 concentration in the soil, maintained for at least several weeks, and the larvae need to be attracted to the capsules to feed on them. In lab experiments we assessed the larval behavior of corn rootworms and wireworms towards these artificial CO_2-capsules. Both pest larvae were attracted by the capsules, but only stayed for short periods at these sites. Thus, additional compounds need to be incorporated into these capsules to increase their attractiveness for the larvae. In German field experiments these capsules, combined with *M. brunneum*, were used in potato fields for wireworm control. Treatments resulted in significantly lower tuber damage in some, but not all fields. Necessary improvements of the attract and kill strategy for anapplication in the field are discussed.

Symposium. Tuesday, 8:40. **60**

Different behavioral responses in specialist and generalist natural enemy interactions (predators and fungi) in a strawberry-mite pest system

Stine Kramer Jacobsen[1], Jørgen Eilenberg[1], Ingeborg Klingen[2], Lene Sigsgaard[1],
[1]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark; [2]Norwegian Institute for Agricultural and Environmental Research (Bioforsk) Plant Health and Plant Protection Division, Høgskoleveien 7, N-1430 Ås, Norway.
Address for Correspondence: stikra@plen.ku.dk

Natural enemies like arthropods and entomopathogenic fungi both contribute to the natural regulation of pests in many crops. As arthropod natural enemies have evolved, they have become a part of a complex multitrophic system and they exist alongside species of entomopathogenic fungi. Some of these entomopathogenic fungi may actually also be a potential threat to arthropod natural enemies. Both arthropod predators and entomopathogenic fungi are important biological control agents of the two spotted spider mite, *Tetranychus urticae* in strawberry. Previous studies on the interactions between these two types of natural enemies show variable results in regards to synergistic/antagonistic effects. We speculated if the degree of specialization of the predator or the fungus could play a significant role. Therefor a behavioral study was conducted to investigate the searching and feeding time of predators (two species tested) in the presence of entomopathogenic fungal spores (two species tested). The predator species used in this study were the generalist predatory bug, *Orius majusculus* and the specialist predatory mite, *Phytoseiulus persimilis*. The entomopathogenic fungal species used was the generalist *Metarhizium brunneum* and the specialist *Neozygites floridana*. Predator behavior was recorded by observations in an experimental setup where the predator was given a choice between two strawberry leaf discs; one with entomopathogenic fungal spores and one without, and both with healthy *T. urticae*. Results suggest that searching and feeding times of both predator species was lower on leaf discs with presence of *M. brunneum* spores compared to no fungal spores. On leaf discs with *N. floridana* spores the searching time of both predators was higher compared to no fungal spores. *O. majusculus* spent more time feeding on prey on the leaf disc with spores of *N. floridana* than on leaf discs without spores, while *P. persimilis* spent less time feeding on the leaf discs with *N. floridana* spores, compared to leaf discs with no fungal spores. Results indicate that the degree of specialization of the beneficial organisms plays a role in the interaction between arthropods and entomopathogenic fungi. Such interactions are important to consider when biological control using several biological control agents is developed.

Symposium. Tuesday, 9:00. **61-STU**

How *Fusarium graminearum* influences insect-plant interactions

Drakulic Jassy [1,2]; Bruce Toby [2], Ray Rumiana [1]
[1] Division of Plant and Crop Sciences, University of Nottingham, UK; [2] Rothamsted Research, Department of Biological Chemistry and Crop Protection, Harpenden, UK
Address for Correspondence: stxjd3@nottingham.ac.uk

Fusarium Head Blight (FHB) disease affects cereals globally, and is caused by a range of toxigenic fungi from the genus *Fusarium*. Wheat (*Triticum aestivum*) is most susceptible to FHB during flowering. The role of insect pests in FHB epidemiology is poorly understood, so the objective of this work was to determine the interactions between the most dominant FHB pathogen, *Fusarium graminearum*, and insect pests that would co-localise on host plants. Grain aphids, *Sitobion avenae*, were used as they are known to colonise wheat ears during flowering. Wheat ears were treated with combinations of fungal inoculum and grain aphids transferred from either healthy or infected previous hosts. Ears treated simultaneously with *F. graminearum* inoculum and aphids incurred significantly higher disease severity, pathogen DNA and accumulation of the mycotoxin deoxynivalenol than ears treated with *F. graminearum* inoculum alone. Olfactometer assays using headspace samples of volatiles from wheat ears inoculated with the pathogen showed that *F. graminearum*-induced volatiles were repellent to aphids. Chemicals responsible for repellency were identified via GC-linked electroantennography and GC-MS followed by olfactometer assays of the electrophysiologically active components. Furthermore, decreased fecundity and survival was observed for aphids fed with *F. graminearum* symptomatic ears. Aphid feeding increased disease progression, therefore benefitted the colonising pathogen, possibly by altering plant defence responses. However, disease induction negatively impacted on aphid survival and reproductive success. Exhibition of a repellent response by aphids to volatiles from diseased plants can be interpreted as an adaptation by aphids to evade the inhospitable enviroment created by the pathogen.

Symposium. Tuesday, 9:20. **62**

Plant-microorganism interactions that shape host-plant selection in the grapevine moth

Geir K. Knudsen[1], Ilaria Pertot[2], Marco Tasin[1,3]
[1]Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway; [2]Edmund Mach Foundation, 38010 San Michele all´Adige, Italy; [3]Integrated Plant Protection, Dep. of Crop Protection Biology, Swedish University of Agricultural Sciences, P.O. Box 102, 230 53 Alnarp, Sweden
Address for Correspondence: marco.tasin@slu.se

Plant-micro-organisms associations may play a role in shaping plant-herbivore interactions. Here, we tested whether the inoculation of a host-plant with a variety of microorganisms would be able to affect the attraction to the plant, the oviposition preference and the fitness of an herbivorous insect. We worked on the system of a phytophagous species (grapevine moth *Lobesia botrana*), its host plant (grapevine *Vitis vinifera*) and the microorganisms associated with the plant. In vineyards, *L. botrana* use a volatile signal to locate the host-plant from a distance and to oviposit on grape. In our experiments, the attraction from a distance and the oviposition preference of the moth were influenced by the microbial activity on the plant. In addition, the quality of the host plant as larval food was importantly changed by the presence of pathogenic or opportunistic microorganisms on the plant. Taken together our results indicated a major role of endemic microorganisms on *L. botrana* host-selection and life-traits. Microbial volatiles appear to be a major cue mediating this kind of interaction.

Symposium. Tuesday, 9:40. **63**

Effect of host plant on aphid susceptibility to the fungal pathogen *Pandora neoaphidis.*

Cezary Tkaczuk[1]; Paresh A. Shah[2], Judith K. Pell[2,3]
[1]Department of Plant Protection, Siedlce University, Siedlce, Poland; [2]Plant and Invertebrate Ecology Department (now AgroEcology Department), Rothamsted Research, Harpenden, UK; [3]Current Address: J.K. Pell Consulting, Luton, UK
Address for Correspondence: tkaczuk@uph.edu.pl

Virulence of the aphid-specific fungus *Pandora neoaphidis*, as

measured in dose-response assays, was compared against the pea aphid, *Acyrthosiphon pisum*, that had been reared on different host plant species. *A. pisum* were reared on dwarf bean then inoculated with *P. neoaphidis* and returned to dwarf bean or inoculated and transferred to field bean, pea or lucerne. The smallest estimated median lethal concentration (LC_{50}) was 7.7 conidia mm^{-2} for aphids returned to dwarf bean, with LC_{50}s of 13.0 and 14.6 conidia mm^{-2} for aphids transferred to field bean or pea, respectively. The largest LC_{50} was achieved when aphids were transferred to lucerne: 2941.0 conidia mm^{-2}. In a subsequent experiment, *A. pisum* were reared on either pea or dwarf bean for four generations before bioassays. The LC_{50} for aphids reared and incubated on dwarf bean was 7.3 conidia mm^{-2}, compared to 13.3 and 15.3 conidia mm^{-2} when aphids were transferred between dwarf bean and pea, or pea and dwarf bean, respectively. The LC_{50} for aphids reared then incubated on pea plants was 27.9 conidia mm^{-2}. Overall, the virulence of *P. neoaphidis* was greatest when *A. pisum* was reared and maintained on dwarf bean, the plant used for long-term routine culturing of the aphid. In conclusion, virulence of *P. neoaphidis* was influenced by host plant and particularly by the plant species to which the host aphid had become adapted. Plant resources may affect the population dynamics of *P. neoaphidis* and could result in a greater impact on aphid herbivores that are not suffering physiological stress related to a change in host plant.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

NEMATODES 2

Contributed paper. Tuesday, 8:00. **64**

Entomopathogenic nematode behavioral responses to chemical cues from cadavers.

Paige Redifer, Brittany Gale, Allison McLain, Glen Stevens, Laura Grochowski
School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, USA
Address for Correspondence: gstevens3@ferrum.edu

Entomopathogenic nematodes (EPN) are exposed to a range of cues in the soil. To the extent these cues are positively associated with the presence of insect hosts, one might hypothesize that EPN would respond positively to such cues. Decomposing animals release many different chemical compounds into the soil, attract large numbers of foraging insects, and produce large numbers of insect larvae. Thus, these chemical compounds may serve as an important cue for foraging EPN. We hypothesized the *Steinernema feltiae* and *Steinernema glaseri* IJs would respond generally positively to two particular compounds (putrescine and cadaverine) produced during animal cadaver decomposition. We further hypothesized that *S. feltiae* would respond more strongly to putrescine, and that *S. glaseri* would respond more strongly to cadaverine. We initially used standard agar-based "bulls-eye" attraction assays, and assessed *S. feltiae* and *S. glaseri* responses to diffusion discs soaked in 5 µl of 50, 100, 500, and 1000 µmol concentrations of each of the two compounds. We followed those agar trials with more realistic small sand column assays, assessing responses to the compounds when they were presented with additional stimuli such as host presence. On agar, responses differed between the different EPN species, chemical compounds, and concentrations, but the chemicals were never attractive and often strongly repellent. Responses were more complex in the sand columns; in particular, the compounds seem to attract more IJs to areas that also contained hosts.

Contributed paper. Tuesday, 8:15. **65**

The *Wolbachia* Endosymbiont as a Nematode Drug Target for Control of Human Filariasis, a Neglected Tropical Disease and Other insect Borne Pathogens

Barton E. Slatko
Molecular Parasitology Group. Genome Biology Division, N
EnglandBiolabs, Inc., Ipswich MA USA
Address for Correspondence: slatko@neb.com

Most human filarial nematode parasites and arthropods are hosts for a bacterial endosymbiont, *Wolbachia*. In filaria, *Wolbachia* are required for normal development, fertility and survival, whereas in arthropods, they are largely parasitic and can influence development and reproduction, but are generally not required for host survival. Due to their obligate nature in filarial parasites, *Wolbachia* have been a target for drug discovery initiatives using several approaches including diversity and focused library screening and genomic sequence analysis. *In vitro* and *in vivo* anti-*Wolbachia* antibiotic treatments have been shown to have adulticidal activity, a long sought goal of filarial parasite drug discovery. In mosquitoes, it has been shown that the presence of *Wolbachia* can inhibit the replication of certain viruses, such as Dengue, Chikungunya, Yellow Fever West Nile, and the infectivity of the malaria-causing protozoan, *Plasmodium* and filarial nematodes. Furthermore, *Wolbachia* can cause a form of conditional sterility that can be used to suppress populations of mosquitoes and additional medically important insects. Thus *Wolbachia*, a pandemic endosymbiont offers great potential for elimination of a wide-variety of devastating human diseases.

Contributed paper. Tuesday, 8:30. **66**

Differential PirAB expression of the entomopathogenic bacterium *Photorhabdus luminescens* (Enterobacteriaceae) based on tissue association and portal of entry to the insect host

Anaïs Castagnola[1,2]; Nathaniel Davis[3]; Belen Molina[4]; S. Patricia Stock[1]; John G. McMullen II[1]
[1]Department of Entomology, University of Arizona; [2]Center for Insect Science, University of Arizona; [3]Pima Community College; [4]Department of Ecology and Evolutionary Biology, University of Arizona
Address for Correspondence: anais@email.arizona.edu

Photorhabdus bacteria gain access to an insect host by their association with the free-living infective juvenile stage (IJ) of *Heterorhabditis* nematodes. Penetration of the insect can be achieved through three different portals of entry: a) digestive (mouth, anus), b) tracheal (spiracles) and c) integument. Studies have shown that *Photorhabdus* may colonize other tissues before they establish in the insect's hemocoel, the final destination for full release of bacterial symbionts and completion of their life cycle. It is likely that *Photorhabdus* employs effectors related to virulence factors in pathogens for adhesion, invasion, and intracellular growth in its host's cells. In this study we investigated tissue aggregations and virulence factors by measuring *Pir*AB toxin expression of *Photorhabdus luminescens* (TT01) in different insect tissues and concurrent to different portals of entry used by their nematode hosts.

Contributed paper. Tuesday, 8:45. **67 STU**

Candidate Virulence Loci in Pan-Genome of the Entomopathogenic Bacterium, *Xenorhabdus bovienii* (Gamma-Proteobacteria: Enterobacteriaceae)

John G McMullen II[1]; Gaelle Bisch[2]; Jean-Claude Ogier[2]; Sylvie Pagés[2]; Sophie Gaudriault[2]; S. Patricia Stock[3]
[1]University of Arizona, School of Animal and Comparative