ALTERNATIVE STRATEGIES OF PLANT PROTECTION AGAINST INVASIVE INSECT PESTS

MINI SYMPOSIUM

BOOK OF ABSTRACTS

28 September 2022

Jožef Stefan International Postgraduate School Lecture Room, Jamova 39, Ljubljana

Mini symposium

Alternative strategies of plant protection against invasive insect pests

Organizer

Department of Biotechnology, Jožef Stefan Institute

Venue

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Introduction

Transcontinental trade and human travel, as well as climate change, are leading to an ever-growing problem with invasive alien species. These pose a serious threat to ecosystem function, biodiversity, and agriculture. Lacking natural enemies in their new environment, they spread rapidly and can often only be controlled by the massive use of chemical pesticides, many of which are banned in Europe, leaving few options for controlling invasive pests. Therefore, there is an urgent need for new alternative pest control options that can be used as part of a climate-smart integrated pest management approach. This mini-symposium will present several of these approaches, including the use of insect sex pheromones, entomopathogenic nematodes, and fungal proteins with highly specific toxicity. An important aspect of developing new alternative pest management strategies is to consider the effects on non-target species and understand the mechanisms of action of new biopesticides, which can greatly improve the application method and dosage for optimal efficacy. In this way, we can develop new strategies for environmentally friendly and safe biopesticides for sustainable agriculture in a changing climate and under pressure from invasive species.

The mini-symposium is organised within the framework of the project "Novel fungal proteins as biopesticides for control of challenging invasive alien agricultural pests -FunContrAPest-", jointly funded by the Slovenian Research Agency (ARRS, J4-2543) and the Hungarian National Research, Development and Innovation Office (NKFIH, 134356 SNN20). In addition to project members from the Plant Protection Institute and the Institute of Mathematics and Basic Science of the Hungarian University of Agriculture and Life Sciences (Dr. Stefan Toepfer, Szabolcs Toth, Dr. Marta Landanyi), the Agricultural Institute of Slovenia (Dr. Jaka Razinger, Dr. Špela Modic, Dr. Maja Smodiš Škerl) and from the Biotechnical Faculty of the University of Ljubljana (Assist. Prof. Dr. Nada Žnidaršič), we also invited Assist. Prof. Dr. Špela Babeler from the National Institute of Biology and Prof. Kristina Sepčić from the Biotechnical Faculty of the University of Ljubljana to present their complementary work on alternative crop protection strategies.

Dr. Jerica Sabotič
Organizer and FunContrAPest project leader

Programme

Towards sustainable bioproduction of pheromones for insect pest control in agriculture

Špela Baebler

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To lower the environmental burden of pest control, insect sex pheromones have been introduced as an alternative plant protection agent, as they provide a sustainable and species-specific regulation of insect populations. Nevertheless, their widespread use is limited due to unsustainable and cost-ineffective manufacturing by chemical synthesis. Thus, biomanufacturing in plants is a promising alternative.

Recently developed SexyPlants, a transgenic *Nicotiana benthaminana* plants producing a blend of moth (Lepidoptera) pheromones, exhibit growth and developmental penalty, diminishing the potential for commercialization. To gain insight into the underlying molecular responses, we analysed the whole-genome transcriptome and evaluated it in relation to growth and pheromone production in low and high producing transgenic plants. Differential gene expression analysis revealed vast transcriptional reprogramming in high producing plants, indicating that the observed growth penalty was not solely a consequence of a higher metabolic burden imposed upon constitutive expression of a heterologous biosynthetic pathway, but rather the result of signalling pathways perturbation.

In contrast to moth pheromones, enzymes producing irregular terpene species-specific mealybug (Coccoidea) pheromones are not known. To elucidate pheromomone-producing pathway in citrus mealybug (*Planoccoccus citri*), we have compiled transcriptome (RNA-Seq and Iso-Seq) data. Combining transcriptome with genomic data and functional annotations, we have identified 39 candidate genes for isoprenyl diphosphate synthases (IDS) that were tested functionally in bacterial and plant expression systems. From those, 5 showed regular but only one candidate showed weak regular IDS activity. Further studies are underway to identify this crucial component of *P. citri* pheromone producing pathway.

Nematode-based biological control solutions against invasive insect pests

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The arrival of alien insect species in new regions often leads to invasions as their specific natural enemies have been left behind in their area of origin. Due to the lack of natural enemies, synthetic pesticides are the major control option. The influx of pesticides into the agro-ecosystem can be enormous, and less disruptive control options are needed. One such option is the use of entomopathogenic nematodes (EPN). Although being largely beneficial worms in the soil environment, they can be used against below- as well as above- ground insect pests. An example for below ground usage of EPN is the management of larvae of the maize pest *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae). However, particularly above-ground insect pests are usually highly susceptible to EPN as they have not co-evolved next to each other. Prominent examples are larvae of the tomato pest *Phthorimaea* (*Tuta*) *absoluta* (Lepidoptera: Gelechiidae) or the maize pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae). As EPN are sensitive to abiotic stress, application technologies need to be carefully developed fitting the EPN, the pest and local conditions. Here we present 15 years of research on the development of practical and effective application technologies for EPN. Our findings clearly demonstrate that EPN are excellent tools for the biological control of invasive insect pests once the application technology has been developed.

We acknowledge funding from ARRS Slovenia (J4-2543) and NKFIH Hungary (SNN 134356), the NCST-NRIF-IDRC/SSR-AGR/002/2021 Rwanda, and the donors of CABI`s Action on Invasives and Plantwise+ programs www.cabi.org/about-cabi/who-we-work-with/key-donors.

Experiments on formulations and application techniques of entomopathogenic nematodes most suitable for the control of western corn rootworm, *Diabrotica v. virgifera* and the fall armyworm, *Spodoptera frugiperda*, in maize, as well as against tomato leaf miner, *Tuta absoluta* in tomato(Photos: CABI, photo of gels by Patrick Fallet, Univ. Neuchatel

Insecticidal activity of protein complexes from oyster mushroom on western corn rootworm

Kristina Sepčić

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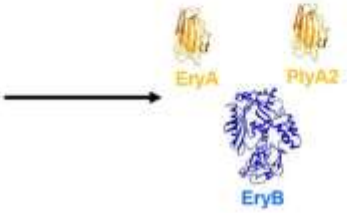
Ostreolysin A6 (OlyA6), a 15 kDa protein produced by the oyster mushroom (*Pleurotus ostreatus*), binds to membrane cholesterol/sphingomyelin domains and, together with a larger protein partner pleurotolysin B (PlyB) co-produced by the same organism, forms 13-meric transmembrane pore complexes. Further, OlyA6 can bind even 1000-times more strongly (KD ~ 1 nM) to an insect-specific membrane sphingolipid ceramide phosphoethanolamine (CPE) and, in concert with PlyB, exert potent and selective insecticidal activity against two economically important coleopteran pests: the Colorado potato beetle and the western corn rootworm (WCR). We have used cryo-electron microscopy to further explore the mechanism of membrane interaction of OlyA6/PlyB complexes with lipid vesicles composed of artificial lipids containing CPE, and with WCR brush border membrane vesicles. In both lipid preparations, the formation of multimeric transmembrane pores, similar to those described on cholesterol/sphingomyelin membranes, was observed. We have also analysed the histological alterations of columnar epithelium comprising the midgut wall of WCR larvae fed with OlyA6/PlyB mixture, including vacuolisation of cytoplasm, swelling of apical cell surface into the gut lumen, and delamination of basal lamina underlying the epithelium. Our combined results strongly suggest that the molecular mechanism of insecticidal action of OlyA6/PlyB arises from the specific interaction of OlyA6 with the invertebrate-specific membrane lipid receptor, and consequent formation of transmembrane pores in the insect midgut. This mode of membrane binding is different from those described for similar aegerolysin-based complexes of bacterial origin (e.g., Cry34Ab1/Cry35Ab1), or other *B. thuringiensis* Cry toxins, which associate with protein receptors.

Pleurotus mushrooms produce aegerolysins and proteins with MACPF-domain

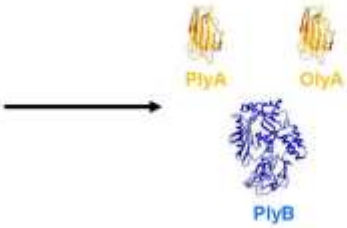
Pleurotus eryngii



OlyA and PlyB form transmembrane pores in artificial membrane vesicles composed of selected **sphingolipids** and **cholesterol**

Lukoyanova et al., 2015, *PLoS Biology*

Pleurotus ostreatus

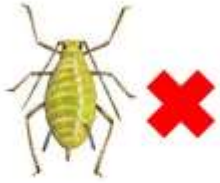


Tested plant pests

Drosophila suzukii

Galleria mellonella

Leptinotarsa decemlineata
Colorado potato beetle
LD_{50} = 9.0 μg/cm²



Sitobion avenae

Tenebrio molitor

Diabrotica virgifera virgifera
Western corn rootworm, **WCR**
LD_{50} = 7.4 μg/cm²

Challenges in controlling the maize pest *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae) under field conditions

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Diabrotica v. virgifera LeConte has been invading Europe since the 1980s and is causing serious damage to the maize production (*Zea mays* L.). The main control option against this pest is crop rotation breaking the pest's life cycle. In continuous maize growing, farmers have been using insecticidal seed coatings, soil insecticides or entomopathogenic nematodes. However, control effects of those methods appeared highly variable, and reasons behind variability are often unknown. Therefore, we collected multiple-year and site data on the field efficacy of several treatments at reducing pest populations and protecting the maize roots, as well as data from 32 biotic and abiotic factors. Experiments took place in Hungary between 2010 and 2020. Firstly, we analysed whether the cause of the observed variability in efficacies may originate from temporal effects of treatments during the cropping season. Astonishingly, results showed that treatments, regardless of soil insecticides or entomopathogenic nematodes, remain effective across the entire season of larval stages in the soil. Secondly, we analysed whether any of the recorded abiotic or biotic factors may affect efficacies. Despite a number of minor trends, there seem no key factor having a major effect on the treatment efficacies across locations and years. Particulaly none of the studied factors seem to negatively affect the performance of entomopathogenic nematodes. In conclusions, it still remains largely unknown why synthetic pesticides or biocontrol agents used in the maize agro-ecosystems lead to sometimes variable effects, something that needs to be further studied.

The studies were funded by the CTI program of BWL Switzerland, the LTZ Baden Wuerttemberg of Germany, the LfL of Bavaria of Germany, a Hungarian state PhD scholarship (magyar állami ösztöndíj) (2018-2022), CABI`s core donors (https://www.cabi.org/about-cabi/who-we-work-with/key-donors/) and the National Excellence Program of the Ministry for Innovation and Technology under the National Research, Development and Innovation Fund (ÚNKP-20-3-II-SZIE-44).

Root damage assessment in one of the numerous experimental maize fields, heavily infested by larvae of the western corn rootworm, *Diabrotica v. virgifera*, Hungary (Photo: CABI)

Analysis of the competition between aphid species *Aphis pomi* DeGeer and *Aphis spiraecola* Patch

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The native, non-host-alternating green apple aphid (*Aphis pomi* DeGeer) and the invasive, host-alternating green spirea aphid (*Aphis spiraecola* Patch) are serious pests of apple, often forming mixed colonies in the canopy of apple trees in Europe. Although some studies suggested the presence of some kind of competition between the two species feeding at the same ecological niche, which can lead to the competitive displacement of the native species by the invader under certain conditions, the presence and nature of such a competition have not been revealed.

In an international project, green aphid samples were collected from 11 regions of Europe (United Kingdom, Denmark, Asturias, Catalonia, France, Italy, Poland, Czech Republic, Slovakia, Hungary and Kosovo), representing the distribution and performance of the two species across the continent. The sampling was executed in the growing seasons of 2018 and 2019, with four sampling dates (May, June, July and September) from two orchard pairs (two organic and two IPM orchards) from each location following the same protocol. At one sampling date, aphid individuals were collected from 33 colonies from each experimental orchard (3 individuals from 'estimated colony size under 100', and 20 individuals from 'estimated colony size over 100' colonies); the estimated colony size, the shoot growing intensity and the density of the aphids in the orchard were recorded as background data. The aphids were identified by species level and the length of the hind tibia of the individuals was measured as a fitness indicator using a stereo microscope (Zeiss Stemi 2000). We provide some new statements based on hind tibia sizes of the two species depending on colony size and abundance rates together with our approaching modelling steps of investigation. We also show the limitations of our findings.

Entomotoxic lectins screened on *Halyomorpha halys* [Stål] in artificial diet-based bioassays

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The brown marmorated stink bug, *Halyomorpha halys* [Stål] (Hemiptera: Pentatomidae), is a harmful insect pest native to eastern Asia and invasive to Europe, North and South America. This highly polyphagous species feeds on over 300 different host plants, including many economically important field, fruit and vegetable crops. The management of *H. halys* in cultivated crops relies mainly on broad-spectrum insecticides which does not always give a satisfactory control due to the pest's wide host range, high mobility, adaptability and developed insecticide resistance. To control *H. halys* environmentally friendly approach such as application of biopesticides from entomotoxic proteins of higher fungi will be highly appreciated. Therefore, the objective of this study was to evaluate the toxicity and other effects of lectins against the *H. halys* nymphs under standardised conditions. For this purpose, we adjusted artificial (Meridic) diet in which we incorporated the novel proteins. Mortality was assessed over 7 day's period. We report the successful development of the protocol and the results of the screened lectins.

Artificial diet to evaluate the toxicity of *H. halys* nymphs.

Testing toxicity and other effects of novel proteins against *Drosophila suzukii*

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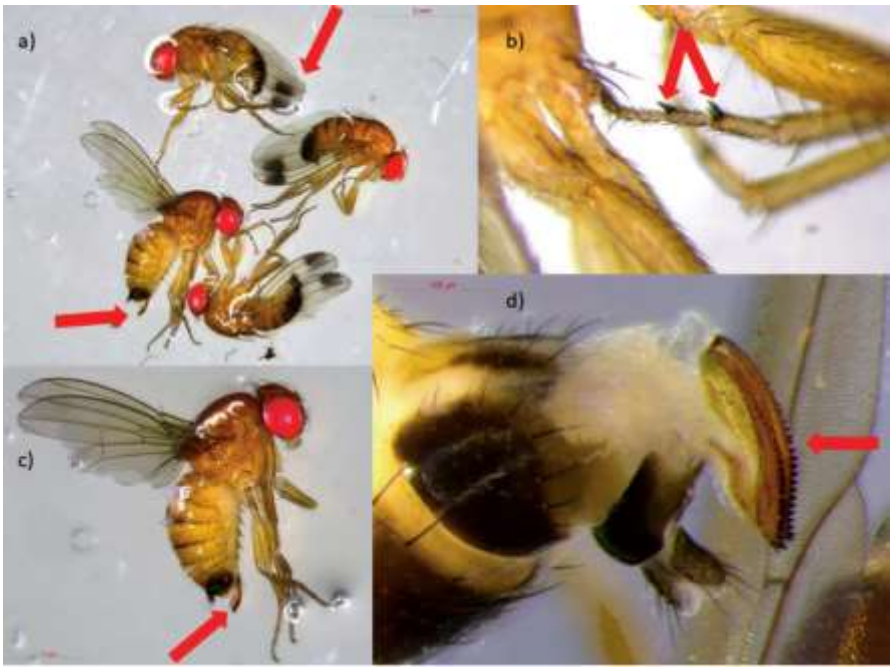
Spotted wing drosophila *Drosophila suzukii* (Matsumura, 1931; SWD; Diptera: Drosophilidae) is a serious pest of soft fruit originating from Asia, which recently invaded North America and Europe. Its excellent adaptation to new climates, extreme fecundity, the absence of natural enemies and no competition for fresh, undamaged fruit speeded up SWD settlement. Unlike other *Drosophila* species, which feed on rotten and damaged fruits, SWD females possess a serrated ovipositor, which enable them to lay eggs into fresh, undamaged fruits causing great harvest losses SWD management is difficult, when one considers the abundance of different fruits available throughout summer and the pest's short generation time. To date various insecticides and protection strategies were evaluated against it. However, many of the tested insecticides cannot be used in organic fruit production and may be disruptive to natural enemies or other beneficial services (agro)ecosystems provide. Accordingly, our research aimed at developing a platform for high throughput screening of potentially toxic proteins from mushrooms, to provide potential innovations to the field of less ecotoxicologically harmful plant protection substances. For this purpose we adjusted artificial SWD diet in which we incorporated the novel proteins. We report the successful development of the protocol and the results of the so far screened lectins.



Recognition of the spotted wing drosophila. a) Males have a dark spot on the tip of the wings. b) Tarsomere i and ii of male forelegs bear a set of spines each (sex combs). Females (c) have clear wings, and a strongly sclerotized ovipositor with black teeth (d). (Photos: Jaka Razinger).

Toxicological studies on honey bees (*Apis mellifera carnica*)

Maja Ivana Smodiš Škerl

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Honey bees *(Apis mellifera)* are one of the most important pollinators of cultivated crops and wild vegetation, and therefore vulnerable to pesticide-induced impacts. The losses of honey bees caused by environmental pollutants suggests that other beneficial insects may experience a similar outcome. Here we describe two methods used for studying pesticide or chemical toxicity towards *Apis mellifera*. With the first test we treat adult bees (*Apis mellifera carnica*) and determine acute toxicity of selected novel proteins in 24, 48 and 72 hrs. The purpose of our second test is to determine the chronic toxicity to honey bee larvae after a repeated oral exposure before pupation. At the end of experiment, we determine mortality level and weight of larvae. Next, we will detect the level of cell death in the larval midgut, and search for potential sub-lethal changes in the tissue. These methods are very useful in risk assessment schemes and in scientific research with the main goal to determine and explain acute and chronic effects of any target compound on *A. mellifera*.

Left – Acute toxicity testing with adult honey bees (*A. m. carnica*) in May 2022. Right – Our latest artificial rearing of larvae (*A. m. carnica*) and chronic toxicity testing with novel proteins at the Agricultural Institute of Slovenia.

Insect midgut – functional ultrastructure from the perspective of new crop protection strategies

Žnidaršič Nada[1], Bogataj Urban[1], Mrak Polona[1], Žigon Primož[2], Razinger Jaka[2], Sabotič Jerica[3]

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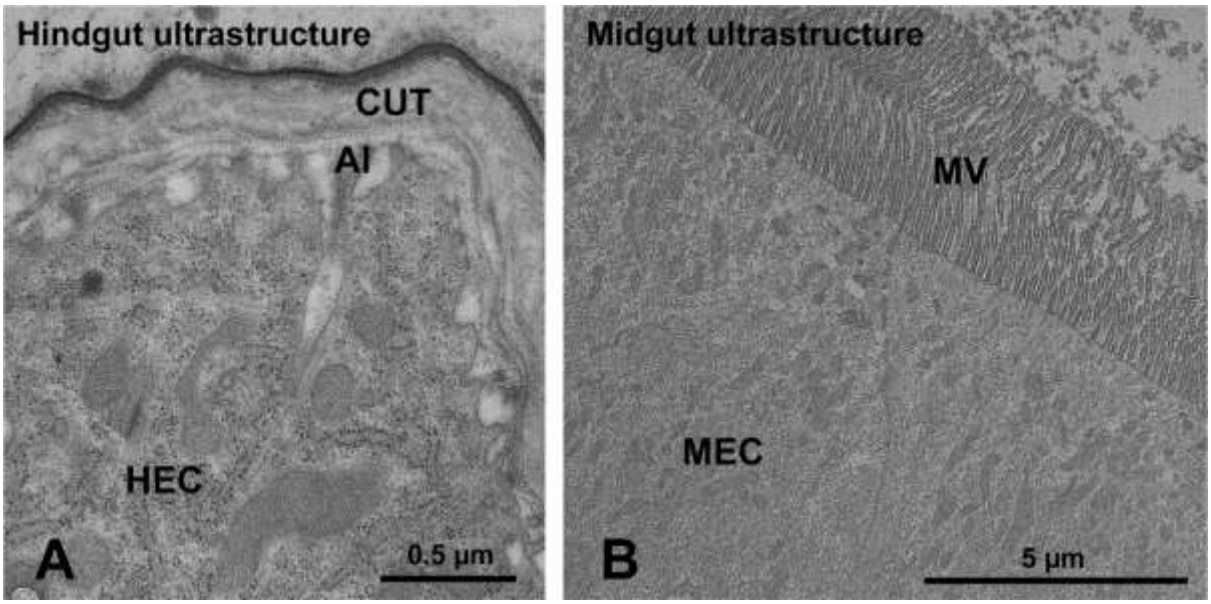
The insect gut is positioned at the interface between the external and internal environment and comprises three main anatomical and functional regions: foregut, midgut and hindgut. The foregut and hindgut are ectodermal in origin and they are covered by a chitinous cuticle on the luminal side, while the midgut derives from endoderm and it is not lined by the cuticle. The midgut epithelium plays a central role in digestion, nutrient absorption and protection against toxins and pathogens. Additional level of complexity relates to variation of cell morphology and physiology along the midgut anterior-posterior axis, that is explained in detail for *Drosophila melanogaster*, but has not been clarified in other species. A better understanding of midgut functional ultrastructure during animal development and in stress conditions is needed to enable formulation of new strategies for crop protection. Effects of xenobiotics are frequently reported as reduction of survival, mass reduction and delay or prolongation in development. Microscopic analyses enable the evaluation of the effects on the development and on the insect morphology and structure at different scales ranging from the whole organism to tissue and cell level. Our current work focuses on the characterization of midgut architecture in two insect species that are important from the perspective of crop protection, spotted wing drosophila (*D. suzukii*) and Colorado potato beetle (*Leptinotarsa decemlineata*).



Ultrastructure of distinct gut regions in Colorado potato beetle larva. A) Apical part of hindgut epithelial cell (HEC) is lined by cuticle (CUT) and the apical plasma membrane forms apical invaginations (AI). B) Apical part of midgut enterocyte (MEC) displays a prominent brush border of microvilli (MV).

List of invited speakers

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