IOBC / WPRS

12th meeting of the Working Group "Pesticides and Beneficial Organisms"

Zadar (Croatia)

30th September - 4thOctober 2018

BOOK OF ABSTRACTS

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University of Zadar

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Abstracts

O1

Registration process, IPM and Beneficial arthropods other than bees: Do we assess the right species? An analysis from the IOBC database

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Abstract: The IOBC database has been implemented to list all public data that concern the possible effects on pesticides on beneficial arthropods, obtained with methods that follow IOBC standards: data from IOBC activities, scientific publication and studies performed in the context of the pesticide registration process at the European level. The database includes over 7500 entries, with about 50% coming from the pesticide registration process, on a limited number of indicative species.
The IOBC database can be used to establish product ecotox profiles, compare pesticides programs, establish selectivity lists for IPM and also to compare the sensitivity of different species, for which a sufficient number of data is available. In this context, an analysis was made on the 6-8 basically species used for registration, to determine if we assess the right species for both the registration of pesticides and IPM development.
Aphidius rhopalosiphi and *Typhlodromus pyri*, the two standard indicative species can be considered as relatively good indicators for the parasitic wasps and predatory mites groups, respectively. For the plant dwelling predators, *Chrysoperla carnea* and *Coccinella septempunctata* have for most of the products a similar profile, while *Chrysoperla* is often less sensitive than *Coccinella*. However, the other important plant dwelling predators, as hoverflies and predatory bugs could sometimes exhibit large difference in term of sensitivity and only using *Chrysoperla*/*Coccinella* for this group could be hazardous. The same problem occurs with soil dwelling predators, with sometimes important differences between *Poecilus cupreus*, *Aleochara bilineata* and *Pardosa* spp. in term of sensitivity to pesticides. Furthermore, the comparison of the large carabid species Poecilus with smaller carabid as *Bembidion* spp. showed that *Poecilus* is a very robust species and not representative of the risk posed to the whole group.

Keywords: IOBC database, product ecotoc profile, beneficial organisms

O2

Semi-field method to evaluate effects of fresh and aged pesticide residues on adults of the tinny hymenoptera *Encarsia formosa* (Gahan)

Antonio Magaña, Beatriz Dáder, Ángeles Adán, Pedro Del Estal, Flor Budia, Ignacio Morales, Elisa Viñuela, Pilar Medina
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Abstract: *Encarsia formosa* (Gahan) is used worldwide for commercial control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) and it is important in El Maresme region (Barcelona, Spain) in tomato crops.
Adults are tinny in size (1 mm long) and due to the difficulty to handle them, control mortality in trials can be high. This study describes a semi-field method to test effects of fresh pesticide residues in pepper plants on adults of this parasitoid, able to maintain control mortality lower than 7 %. Besides, a comparison of the duration of the pesticide effects when residues were aged inside greenhouse or outdoors under a UV-permeable plastic was done. The method has been validated with water and with acetamiprid (harmful for the natural enemy based on Biobest and Koppert databases), applied at its maximum field recommended concentration in Spain for whiteflies (100 mg/l of active ingredient). Because they have a great influence of the speed of residue degradation we recorded: temperature (ºC), relative humidity (%), UVA, UVB (W/m^2) and PAR radiation ($\mu mol/m^2\ s^1$), illuminance (lux), and light intensity ($\mu\ mol/m^2\ s$).
Lethal effects were evaluated in units consisting of two round ventilated transparent plastic (Glasspack®) cages (0.5 mm thick) connected by a square sponge. Inside the lower cage, a 5 leaves pepper plant (~10 cm high) was introduced. When 50% of females were alive after the 48-h exposure to the residues, the beneficial capacity was assessed in round ventilated plastic cages with a ventilation hole on top covered with a mesh and the floor covered with a piece of wet cotton.
Acetamiprid was harmful (IOBC 4) and persistent (IOBC D) to adults of *E. formosa* (based on high mortality) irrespective if the residues were aged in spring in the greenhouse or in early autumn outdoors.

O3

Application of molecular methods in trophic ecology of useful predators within Mediterranean agriculture

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Abstract: The wide range of molecular methods, such as protein electrophoresis, ELISA, polymerase chain reaction (PCR) and quantitative polymerase chain reaction (qPCR), has been employed in analyzing trophic ecology, in particular eating habits of useful predators and detection of parasitoids within the pest management programs. Furthermore molecular methods are being improved continuously and with very fast pace, and becoming more precise, less time consuming and affordable in ecology studies. In this review we will sum up new achievements and address new perspectives of molecular analyses of trophic interactions applied within Mediterranean agriculture, in particular in olive orchards and vineyards, and the adverse effects of *pesticides* on beneficial predatory arthropods. We will discuss the use of methods within the frame of our project MEDITERATRI (Croatian Science Foundation project), dealing with predator-prey interactions within the Mediterranean Agriculture, with to management types combining Neonicotinoids and Copper as pesticides and their effects on non-target invertebrates.

Key words: molecular methods, trophic interactions, *pesticides*, invertebrates

O4

Pesticide compatibility in commercial pepper and tomato greenhouses when natural enemies are introduced in the nurseries

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Abstract: Successful IPM systems on protected crops use a variety of control measures. However integration of different tactics implies an evaluation of the compatibility of pesticides and natural enemies, as control strategies that only rely on one tactic can fail when pest populations exceed natural enemies' activity or pests become resistant to pesticides.
In this work, we present a two-year trial inside tomato and sweet pepper commercial multispan greenhouses located in Almería, a South-Eastern Spanish region that holds more than 44,000 ha of horticultural protected crops. Experimental blocks were around 72-100 m^2 divided in 4 replicates for monitoring (18-25 m^2), in order to minimize any bias. Growers prefer nowadays to introduce early in the crops some natural enemies (e.g. the mirid *Nesidiocoris tenuis*, the anthocorid bug *Orius laevigatus* and the predatory mite *Amblyseius swirskii*) and they release them in the nurseries. In this work we have evaluated weekly, the compatibility of the most frequently used modern pesticides in the area (chlorantraniprole, flubendiamide, metaflumizone, methyl-chlorpyrifos, spinosad, emamectin benzoate, spirotetramat, sulfoxaflor, pymetrozine, abamectin and methoxyfenozide) with the established populations of the natural enemies cited above. The pests *Tuta absoluta* on tomato, and *Frankliniella occidentalis* and *Bemisia tabaci* on sweet pepper were also followed.
We found that flubendiamide and chlorantraniliprole were the most compatible with *N. tenuis* on tomato, whereas methyl-chlorpyrifos and spinosad cannot be recommended. No differences were found for *T. absoluta* populations the first year but methyl-chlorpyrifos and spinosad significantly reduced moth density the second year. For sweet pepper, chlorantraniliprole and emamectin benzoate were compatible with *O. laevigatus* but not sulfoxaflor. Spirotetramat, pymetrozine and sulfoxaflor statistically decreased *A. swirskii* density. During 2016, *F. occidentalis* density under control conditions was lower than in pymetrozine, spirotetramat, sulfoxaflor and chlorantraniliprole treatments. There were not significant differences for *F. occidentalis* and *B. tabaci* during the second year.

Keywords: Integrated Pest Management, *Nesidiocoris tenuis*, *Orius laevigatus*, *Amblyseius swirskii*, chemical control

O5

Long-term impact of plant protection product mixtures on earthworms in agricultural fields

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Abstract: The environmental risk assessment of plant protection products on soil organisms is mainly based on the outcome of laboratory and extended laboratory studies (EFSA, 2017) while the link from the laboratory to realistic field conditions over several seasons is not well established. Current environmental risk assessment has the focus on single compounds and does not take into account that soil organisms are exposed to a mixture of active ingredients from different plant protection products. In this study, earthworms were sampled at two seasons from eight fields in Croatia and analysed for 300 active ingredients. The concentrations of 26 analysed active ingredients ranged between 0.000 and 0.247 mg/kg earthworm fresh weight with a mean of 0.005 mg/kg earthworm fresh weight. The percentage of samples with values below the limit of detection (LOD), values below the limit of quantification (LOQ = 0.001 mg/kg) and values above LOQ were 29, 42 and 29%, respectively. Based on publicly available draft assessment reports from EC and EFSA, degradation parameters (DT_{50}, DT_{90}) were used to calculate degradation curves and the current concentration in a soil at the date of earthworm sampling. Subsequently, substance-specific concentration factors in soil were established by dividing the analysed pesticide residues in earthworms by the calculated concentrations in soil. The aim of this survey was to check the applicability and reliability of this method by examining: (i) whether the bioconcentration factors calculated in this study are comparable to published bioconcentration factors; (ii) whether mixtures of active ingredients with the same mode of action do pose a risk to the soil earthworm community. Preliminary results show that bioconcentration factors calculated in this study are in a similar range as in the publicly available literature. At least in the case of some fungicides, the "No-Observed-Effect-Concentration" for reproduction on *Eisenia fetida* (Savigny, 1826) is < 1 mg/kg dry soil and may have effects on field earthworm species reproduction for some periods of the growing season.

Keywords: Environmental risk assessment, earthworms, pesticide residues, bioconcentration factors

O6

Revealing an agrochemical synergy and its effect on a biocontrol insect

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Abstract: Hymenopteran parasitoids are effective biocontrol agents, suppressing crop pest populations. However, farmers still apply chemical insecticides to control crop pests, often tank-mixing insecticides with fungicides for simultaneous use. Unfortunately, pesticide risk assessments on biocontrol insects have not accounted for this. We examined the lethal and sublethal effect of combining the neonicotinoid insecticide thiacloprid with the fungicide tebuconazole, using the parasitoid wasp *Aphelinus abdominalis* as a model. We observed synergism between thiacloprid and tebuconazole in combinatory treatments, the effect of some treatments being significantly greater than that of thiacloprid alone, for both mortality and loss of motor control. With this first evidence of lethal and sublethal effects of agrochemical synergism in a biocontrol agent, we show the risk that tank-mixing thiacloprid and tebuconazole imposes on *A. abdominalis* populations, suggesting an urgent need for updating pesticide risk assessment methods regarding non-target organisms, in order to make these risk assessments more field-relevant.

Key words: Hymenoptera, parasitoid, risk assessment, pesticide, neonicotinoid, fungicide

O7

Effect of exclusion nets on spider diversity and composition in IPM apple orchard

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Abstract: Spiders are one of the most abundant natural enemies in apple orchards and have an important role in the biological control of harmful arthropods. Recently coloured shade nets to improve the utilization of solar radiation by fruit trees and to exclude pest species have been introduced in practice. While the coloured netting in apple orchards had a positive effect on fruits, the aim of the our research was to analyse the effects of the nets on spider diversity and species composition. The ground dwelling spider assemblage was sampled by pitfall trapping. Pitfall traps were placed under trees covered by four types of exclusion photo selective nets and uncovered control trees in an untreated experimental plot of an IPM apple orchard. The study was spatially replicated in three rows. During the investigation period 456 individuals belonging to 26 species and 12 families of spiders were collected. Two wolf spider (Araneae, *Lycosidae*) species were dominating the assemblages: *Trochosa robusta* (Simon 1876) and *Hogna radiata* (Latreille 1819) comprised 45% of the total adult catch, and these were the only species that occurred in all treatments. There was no difference either between the abundance, species richness or the species composition of spiders in the treatments. The study strongly indicates that insect exclusion nets have no negative effect on ground dwelling spider assemblages.

Key words: spiders, shade nets, apple orchard, *Trochosa robusta*, *Hogna radiata*

O8

Analysis of pesticide residues in solitary bee-collected pollen in Belgian fruit orchards

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Abstract: Food production heavily relies on insect pollination. Furthermore, fruit production is nearly impossible without pollinators. However, until now little is known about the exposure of solitary bees to pesticides in fruit orchards. Therefore, a field experiment was set up in fruit orchards in different locations in Flanders to assess the risk of solitary bees for pesticide residues. Pollen, weed flower, tree blossom and soil samples were obtained from 10 cherry (*Prunus* spp.) and 8 apple (*Malus* spp.) orchards. Sampling was carried out during the complete adult lifespan of its main pollinators, namely *Osmia cornuta* and *O. bicornis*. We covered the flowering period of cherry and apple (April), as well as the period thereafter (May), when weed flowers (*Taraxacum officinale* and *Bellis perennis*) are the main food source for solitary bees. For the pesticide residue analysis, we used a multi-residue method, that has been validated for more than 100 active substances and employed a QuEChERS extraction followed by separation and quantitation with LC-MS/MS technology. Data are discussed in relation to which active substances are found and whether the concentrations pose a threat to solitary bees.

Key words: solitary bees, *Osmia* spp., pesticide exposure, orchards, LC-MS/MS, multi residue analysis

O9

Literature review of baseline information on RNAi that could support the environmental risk assessment of RNAi-based GM plants

Olivier Christiaens[1], Teodora Dzhambazova[2], Kaloyan Kostov[2], Salvatore Arpaia[3], Mallikarjuna Reddy Joga[1], Isabella Urru[3], Jeremy Sweet[4], Guy Smagghe[1]
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Abstract: As part of an EFSA procurement, a systematic literature search was performed to collect all available peer-reviewed studies on RNAi in invertebrate species (Nematoda, Arthropoda, Mollusca and Annelida) to provide a baseline information on this technology that could support the environmental risk assessment of RNAi-based GM plants. With this literature search, we retrieved a total of 5,076 publications. Based on this database, an overview was compiled of all studies using oral delivery of small RNAs (sRNAs) to these invertebrates. This overview includes information on tested species, life stage, sRNA molecule type, target gene, concentrations used, outcomes, etc. A second part of our assignment was to provide several narrative reviews on different topics such as environmental and cellular uptake of sRNAs, RNAi efficiency and factors involved in sensitivity, possible exposure routes of small RNAs to (non-)target organisms, potential unintended effects by sRNAs on invertebrate species in the agroecosystem and also on the availability and use of genomic data in risk assessment of RNAi-based GM crops. Here, we present an overview of the literature search, the conclusions of the narrative reviews and we provide some comments on information gaps and research requirements.

Keywords: RNA interference, environmental risk assessment, non-target effects

O10

Bioinformatic pipeline to design gene-specific and biosafe dsRNA. What do we have for non-target organisms?

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Abstract: RNA interference (RNAi) is a biological process in which double stranded RNA (dsRNA) molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules. This technology received the Nobel Prize in Physiology and Medicine in 2006.
Today RNAi is employed to control pest insects such as the western corn rootworm *Diabrotica virgifera* via transgenic corn. Also, successes have been reported against other beetle pests as Colorado potato beetle, but also sucking pest insects as Asian citrus psyllids and mites can be used to induce silencing of target genes in insect pests.
While double stranded RNA (dsRNA) can be designed specifically for selected target organisms, non-specific silencing effects, either of the homologue of the target gene or elsewhere in the genome of non-target organisms, can occur if sufficient sequence similarity exists.
In this presentation we will present the bioinformatics pipeline to design gene-specific and biosafe dsRNA. Aspects on available genome data, length and complementarity of dsRNA, mismatches, off-target genes, etc. will be discussed. Together with the theoretical bioinformatics, data, published or from own experiments with non-target organisms, will also be discussed.

Keywords: RNA interference, bioinformatics pipeline, environmental risk assessment, non-target effects

O11

Effect of Eucalyptus globulus Labill., (Myrtaceae) essential oil on non targeted aphidophagous species in a citrus area of the central Mitidja plain (Blidean Atlas, Algeria)

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Abstract: Arthropod beneficials are always affected negatively by devastating pests chemical management, particularly in orchards. At present, plant essential oils with their bioactive natural molecules are promising potential source, because of their antifeedant, repulsive or insecticidal properties. In this work, the temporal effect of *Eucalyptus globulus* (EG) essential oil (EO) formulated solutions (1,5% and 3%) was arrested on non targeted aphidophagous coccinellidae, chrysopidae and syrphidae populations, in an orange orchard situated at Bougara region (Central Mitidja). The efficiency at 3ml/ l, 9ml/ l and 12ml/ l EG essential oil dilutions, was assessed each 48 hours over a 15 days period of exposure from middle of April till the beginning of May 2015, through captures by yellow water traps disposed within the canopies. Recruitment of aphidophagous in the plots was noted after two weeks after treatments. The foliar spraying of EGEO 1.5 % seem to have an unfavorable effect on syrphidae and chrysopidae displacements and a repressive effect of the coccinellidae abundances which remain lower than 2 individuals. In contrast, the repressive effects with the EGEO 3% solutions seem less felt because syrphidae and chrysopidae remain present but with low reported numbers. Coccinellidae predatory species were particularly sensitive to treatments. According to their sensibility, we distinguish a first group consisted essentially of large-sized aphidiphagous ladybirds such as *Harmonia axyridis* associated with syrphidae species *Episyrphus corollae* and *Epistrophe balteatus* which are recruited in low numbers 3 weeks after treatment under the influence of the 3 % formulated EGEO solutions. The second group gathers relatively big size coccinellidae community: *Rodolia cardinalis*, *Harmonia tredecimpunctata*, *Chilocorus bipustulatus*, *Adonia variegata* or small-sized ladybirds as *Clitosthetus arcuatus* associated with *Scaeva pyrastri* and *Sphaerophoria scripta* absent of the orchard for a 15 days period. We note however a good biocenose resumption concerning *Scymnus subvillosus* after 3 weeks of exposure under 1.5 % EGEO treatment. The third group is characterized by the absence of *Rhyzobius lophantae* predatory species of the California red scale *Aonidiella aurantii* after one week of the same formulation application.

Key words: beneficial communities, chemicals, phytopesticides, aphidophagous, *Eucalyptus globulus*, essential oils.

O12

Toxicity of insecticides to *Macrolophus basicornis* (Hemiptera: Miridae), a promising predator of the South American tomato borer *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Abstract: In this study the toxicity of five insecticides on the omnivorous predator *Macrolophus basicornis* (Stal) (Hemiptera: Miridae), which showed a high predation rate of the South American tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) eggs, was evaluated. The highest field recommended concentration of insecticides was used. The lethal effect caused by the insecticides was evaluated by exposing adult predators to residues, in laboratory conditions, in two different surfaces: inert (Petri dishes) and vegetable (tomato leaves). Petri dishes were treated in a Potter precision tower (calibrated pressure: 100 kPa, application volume: 1.5 ± 0.5 mg/cm^2), while leaves were obtained from tomato plants treated with a hand sprayer until runoff (25 mL/plant). Mortality caused by insecticides was also evaluated in greenhouse conditions (treated plants), and the lethal effect persistence was assessed. In treatments that caused low mortalities, the sublethal effect on *M. basicornis* adults' predation rate was evaluated. Adult females were maintained in treated leaves without food during 24h, and after this period each insect was transferred to a Petri dish containing an untreated tomato leaf and 250 *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs. The number of preyed eggs was assessed 12, 24 and 36 h after insects' transfer to Petri dishes. The accumulated number of preyed eggs after 36 h was also recorded. Imidacloprid caused the highest mortality in all tests and kept its toxicity until 23 days after application. Deltamethrin only had a negative effect in case of exposure to fresh residues. Indoxacarb was unsafe in laboratory but it did not show lethal effect under greenhouse. Flubendiamide and spiromesifen residues caused low mortalities to adult predators, however these products reduced *M. basicornis* adults preying rate. Therefore, these products should be used carefully in tomato crops with the predator *M. basicornis*.

Key words: ecotoxicology, insecticide selectivity, residual activity, biological control

O13

Acute toxicity of citrus acaricides to the armored scale predator *Rhyzobius lophanthae*

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Abstract: The coccinellid beetle *Rhyzobius lophanthae* Blaisdell is an important worldwide predator of armored scale species in citrus groves. Laboratory bioassays were carried out to test the effect of acaricides, commonly used in citrus in general, and sulfur pesticides, on acute mortality of *R. lophanthae* larvae and adults. The toxicity of six acaricides (spirodiclofen, fenbutatin oxide, abamectin, summer oil, and two sulfur formulations) were assessed. Acute toxicity was investigated in two situations: (i) direct topical applications and (ii) feeding and contact with dry residues on prey items (oleander scales, *Aspidiotus nerii*). The pesticide abamectin was found to be extremely toxic to *R. lophanthae* larvae and adults in both topical applications and pesticide residue situations. On the other hand, the pesticides spirodiclofen, fenbutatin oxide, summer oil, and two sulfur formulations of various rates (as high as 8-fold the label rate), were found to be harmless to both *R. lophanthae* larvae and adults. Our data suggest that all tested acaricides except for abamectin, are harmless and may be considered compatible with *R. lophanthae*. Furthermore, our data show that *R. lophanthae* was not affected by high doses of sulfur solutions.

Key words: Biological control, Citrus, Pesticide residue, Pesticide toxicity, Topical applications

O14

Effect of imidacloprid and emmamectin benzoate on *Neoscona theisi* (Araneae: Araneidae): An implication for integrated pest management

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Abstract: *Neoscona theisi* is the most abundant orb web spider in many agroecosystems of Pakistan. It plays a vital role in the suppression of insect pests in many crops. However, its population is adversely affected by unselective use of broad spectrum insecticides in the fields. The objective of this study was to determine the influence of imidacloprid and emmamectin benzoate on survival, reproduction and web building behavior of *Neoscona theisi* females under laboratory conditions. Both insecticides were highly toxic at their field application rate. The LC_{50} values of imidacloprid and emmamectin benzoate were 46 and 67 times less than their registered field rates, respectively. The number of egg sacs, weight of egg masses and fecundity rate was significantly low in insecticide treated female than control. The developmental time of progeny also prolonged in insecticide treated spiders. The females treated with insecticides constructed webs with increased mesh height, decreased capture area and increased anomalies than control. Our findings highlight the drastic effects of imidacloprid and emmamectin benzoate on biocontrol potential of *Neoscona theisi*. The utilization of these insecticides in agriculture needs risk assessment for different functional groups of organisms residing agroecosytems.

O15

The anti-fungal mechanism of glycolipids from *Dacryopinax spathularia* and its potential application in chili anthracnose disease control

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Abstract: In a search for a substitute for chemical fungicide against chili anthacnose disease, an extract from liquid fermentation of the jelly mushroom *D. spathularia* was found to suppress spore germination and mycelial growth of *Colletotrichum capsici* (CC), *C. gloeosporiodes* (CG) and *C. acutatum* (CA). Further characterization revealed that the extract exhibited broad anti-fungal activity against various fungal pathogens with the minimal inhibitiory concentration (MIC90) of 6.25 microgram/ml or below, but no toxicity towards mammalian cells. Its efficacy against anthracnose infection in chili pepper (*Capsicum annuum*) was preliminarily evaluated using attached fruit assay, where spray application of extract at 125 microgram/ml reduced the incidence and severity of CA infection by 50 and 60%, respectively. Since the extract was mainly composed of glycolipids which presummably had biosurfactant property, its effect on fungal cell membrane was examined using membrane staining fluorescence dye FM4-64 and confocal laser scanning microscope.

We found that germinating CA spores displayed deformity and stained intracellularly after 10 min exposure to 10 microgram/ml extract, suggesting the lost of cell membrane integrity. The treated CA germlings were completely damaged after 30 min, in contrast to the untreated germlings whose plasma membrane stayed intact until 60 min.

The membrane damaging effect of this extract was confirmed in a separate experiment using CG strain expressing green fluorescent protein, where the leakage of cytosolic content was seen after 10 min exposure to the same amount of extract. The extract did not affect fungal cell wall, as its anti-fungal activity was not altered in the present of an osmo-protectant. Due to its mechanism of action, which is unlikely to induce resistance among fungal pathogen, and potential for scale-up production, the glycolipid-rich extract from *D. spathularia* culture offer an appealing option for plant fungal disease management.

Key words: anti-fungal, anthracnose, *Dacryopinax spathularia* and glycolipid

O16

Influence of microbiota in the susceptibility of parasitic wasps to abamectin insecticide: deep sequencing, esterase and toxicity tests

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Abstract: The parasitic wasps *Eretmocerus mundus*, *Eretmocerus eremicus* and *Encarsia formosa* are important natural enemies of whiteflies. A broad understanding of their biology, ecology and behavior has been achieved, but the composition and role of their microbiota is not fully determined. The knowledge of the bacteria present in insects might be useful to manage species of human concern such as natural enemies or pests. Here, we assessed the microbiota present in adults of *E. eremicus*, *E. formosa* and two strains of *E. mundus* by MiSeq® 16S rRNA amplicon sequencing. *Staphylococcus*, *Streptococcus*, *Rothia*, *Arthrobacter* and *Pasteurellaceae* were detected in all the studied insects and their potential beneficial functions for the parasitic wasps are discussed. Moreover, enzymatic, antibiotic and residual contact tests were done to determine the influence of *Arthrobacter* species in the susceptibility of *E. mundus* to pesticides. The results suggest that this bacterial genus can have an influence on the toxicity of *E. mundus* to pesticides.

Keywords: *Eretmocerus mundus*, *Eretmocerus eremicus*, *Encarsia formosa*, deep sequencing, pesticide susceptibility, *Arthrobacter*

O17

Potential vectors of bacterium *Xylella fastidiosa* in olive growing area of Croatia

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Abstract: The appearance of the bacterium *Xylella fastidiosa* in Europe and its role in olive degradation have influenced on faunistic research of insects under order Hemiptera. Special emphasis is given on the species belonging to families Cicadidae, Aphrophoridae, Cercopidae and Cicadellidae under suborder Cicadomorpha which are considered as potential vectors and can transfer this bacterium from infected to healthy plant. This paper contains results of faunistic research conducted during the year of 2015. A total of 701 individuals were captured within Cicadomorpha and 15 within Fulgoromorpha suborders. They were collected on natural habitats of different grasses, on meadows and natural weed vegetation in olive orchards in Croatia. One of the most widespread potential vector species *Philaenus spumarius* were collected for carrying out molecular analyses to detect the infected individual. The results of the PCR analysis showed the absence of bacteria in the analysed insects. According to high phytosanitary risk of this disease and the possibility of spreading in the olive region of Croatia it is necessary to continue monitoring potential vectors, which is essential factor in the early detection of influenced insects and can effect on further phytosanitary measures.

Keywords: *Xylella fastidiosa*,potential vectors,Cicadomorpha, *Philaenus spumarius*, olive

O18

Biocontrol organisms against fire blight (*Erwinia amylovora*) in pear flowers: population dynamics and effect on infection

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Abstract: Fire blight remains an important threat to apple and pear orchards throughout temperate zones. When conditions are favorable during bloom with warm weather and frequent rain, flowers are at serious risk of infection by the causal bacterial pathogen *Erwinia amylovora* spreading from overwintering cankers. As curative measures are limited, preventing these infections is of key importance. Biocontrol organisms (BCOs) can have an antagonistic effect on *E. amylovora* population buildup in the flower. By delaying this epiphytic phase of *E. amylovora* flower infection, BCOs can prevent the pathogen from entering the flower through the nectarthodes in the hypanthium. However, BCO populations in the flower need to be established before *E. amylovora* appears. This report details the population dynamics of BCO's when sprayed on pear flowers as well as the interaction with E. amylovora development in the flower and with the development of infection symptoms.
Based on their in vitro inhibition of E. amylovora growth, a Pseudomonas strain represented in the pear flower microbiome and a soilborne Bacillus strain were selected. The BCO *Aureobasidium pullulans* strains DSM 14940+14941 present in the registered product Blossom Protect® (Bio-Ferm, Tulln, Austria) were also included in the tests in the commercial formulation. All BCO's displayed growth in pear (*Pyrus communis* cv. 'Conference') flowers under both warm and cold circumstances, and reduced *E. amylovora* population buildup in the flowers to a similar degree. However, only A. pullulans significantly reduced flower infection symptoms. No synergistic effect between A. pullulans and one of the other BCO's on symptom reduction was detected. We propose that differences in mode of action and flower niche preference explain the efficacy of BCO's in preventing *E. amylovora* infection. Results are discussed within the broader framework of developing an approach for BCO application by flower spraying followed by secondary dispersal by bee pollinators.

Key words: *Erwinia amylovora*, fire blight, Biological Control Organism, *Aureobasidium pullulans*, population dynamics

P1

Fluctuating asymmetry of shape as a bio-indicator tool to detect levels of disturbance between agricultural productions

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Abstract: Carabid beetles are often used as indicators of habitat change due to their fast response to environmental changes, well-known biology and ecology, as well as requiring easy sampling methods. Agricultural practices in intensive agricultural production often create a modified environment, which generates different degrees of stress in insects. The tendency of a development system to produce morphological modifications as a response to random perturbations is usually called developmental instability or developmental noise. One of the most common techniques applied to analyse the impact of developmental instability on a particular morphological feature is fluctuating asymmetry (FA). This study assessed the impact of two different integrated agro-ecosystems a) perennial (orchard) and b) annual (arable) crops on the carabid *Pterostichus melas melas* (Creutzer, 1799) morphology, by using fluctuating asymmetry (FA) as biomonitor of developmental stability. Shape variation and FA levels were estimated using geometric morphometrics. The results obtained using geometric morphometric analyses such as regressions (FA scores vs Shape) and partial least squares showed that carabids that inhabited the perennial agro-ecosystem seem to have adapted to the strong anthropogenic influence at the phenotypic level, while the carabids inhabiting annual agro-ecosystems experience more unstable environments and their phenotypes seem to have been change more recently. It was expected that phenotypes of the annual agro-ecosystems would be more variable than the long-established ones. Different agricultural agro-ecosystems generate different disturbance degrees in insect communities, and these effects can be successfully quantified by applying geometric morphometric techniques.

Key words: *Pterostichus melas melas*, fluctuating asymmetry, geometric morphometrics, agro-ecosystem

P2

Helicoverpa armigera (Hübn.) feeding preference for crop and weed plants

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Abstract: *Helicoverpa armigera* (Hübner, 1808), the cotton bollworm, is a highly polyphagous pest whose caterpillars feed primarily on crops, but can attack the weed plants in absence of food. The massive occurrence of this pest was recorded in the fall of 2016 in Lukač on the weed ambrosia. Feeding preference tests were carried out in laboratory in order to determine if ambrosia was preferable to oats, beans and sugar beet, crops on which this pest is important and common. For all test plants the amount of eaten leaves was measured, as well as the leaf surface, and the results were analyzed by ANOVA. If all the tested plant species ("choice test") were offered at the same time, the cotton bollworm caterpillars significantly preferred the ambrosia plants. If only one plant species in the test was offered ("no-choice test"), the cotton bollworm caused more damage on ambrosia, which was significantly higher only when compared to damages on the bean plants. In contrast to the damage, the "no-choice test" showed that cotton bollworm caterpillars had eaten significantly more ambrosia leaf suface than other plants in the study. Feeding preference tests have shown that the weed ambrosia is very acceptable plant host to cotton bollworm and that, in the absence of cultivated plants, it may serve as an alternative source of nutrition, thereby ensuring survival when crops are unavailable. This feeding preference study was made for the first time on cotton bollworm in Croatia. Therefore, it is recommended to carry out additional feeding preference tests on other plant species or investigate egg laying preferences of the cotton bollworm.

Key words: ambrosia, feeding preference tests, *Helicoverpa armigera*

P3

Risk assessment of microbial preparations on beneficial insects (*Bombus terrestris*)

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Abstract: Microbial preparations have become more popular during the last decade as part of an IPM strategy. Despite being more environmentally friendly compared to traditional chemical pesticides, these microbial preparations still carry a risk to infect not only the target species, but also beneficial arthropods such as natural enemies and pollinators.
In this paper we report on lab assays where microbial preparations were exposed to workers of bumblebees (*Bombus terrestris*) as a dry powder formulation. *B. terrestris* is an important generalist pollinator of wild plants and many agricultural crops worldwide. However, such dry conditions may not always be in line with field conditions. One crucial difference is that the conditions under lab assays with dry powder may not facilitate germination of the spores. However, under realistic field conditions, one could expect spore germination to present a risk for both individuals and the colonies. Our results demonstrated that the traditional lab assays cannot consider these dynamics between bumblebees and microbial preparations without extra tests, and so we highlight here the importance of including analysis of spore germination risk.

Key words: Microbial preparations, Powder, Lab assays, Risk assessment, Bumblebees, Integrated Pest Management

P4

Risk assessment of dimethoate in solitary bees. Experimental set-up and what do we learn for pollinators in general?

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Abstract: Nowadays, the toxicity testing of plant protection products (PPPs) is limited to two pollinators, namely the honey bee (*Apis mellifera*) and bumble bee (*Bombus terrestris*), despite the great importance of solitary bees and stingless bees in crop pollination. Furthermore, there is no evidence that sensitivity to PPPs of non-*Apis* bees is comparable with that of honey bees. The objective of this research is to contribute to a reliable assessment factor. This initial research uses a first-tier acute contact test protocol developed by the ICP-PR Non-*Apis* Working Group with *Osmia bicornis* as test organism and dimethoate as test substance. It is discussed whether other species are sufficiently protected when *A. mellifera* is used as surrogate organism in environmental risk assessment of PPPs.

Key words: solitary bees, *Osmia bicornis*, pesticide exposure, contact toxicity, LD_{50}, plant protection products

P5

Assessing the safety of a dsRNA targeting the Colorado potato beetle, to the predator *Chrysoperla carnea*

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Abstract: RNA interference (RNAi) can be used to induce silencing of target genes in insect pests. While double stranded RNA (dsRNA) can be designed specifically for selected target organisms, non-specific silencing effects, either of the homologue of the target gene or elsewhere in the genome of non-target organisms, can occur if sufficient sequence similarity exists. Furthermore, unintended effects due to non-sequence dependent mechanisms (e.g. immunostimulation or saturation of the RNAi machinery) have been hypothesized as well. Off-target effects have been most commonly observed in taxonomically related species, though they can occur even in different insect orders. Since not all insect species show similar sensitivity to dsRNA, the assessment of the selectivity of specific dsRNA during the development of dsRNA-based pesticides or resistant plants is a very important goal of the process.
In this paper, in order to assess the selectivity of a dsRNA targeting *Leptinotarsa decemlineata* Say clathrin gene, we conducted laboratory experiments using the larvae of the generalist predator *Chrysoperla carnea* Stephens as non-target species, for which very little genomic data exist. Second instars of the predator were fed with a sucrose solution containing dsRNA at a concentration about 200 times higher than the one which proved to be lethal for *L. decemlineata*. Different types of food supply were tested and in order to mimic natural conditions, a mixed diet constituted of sugar solution and arthropod eggs was used. The data are discussed in terms of lethal and sub-lethal effects.

Keywords: RNA interference, environmental risk assessment, non-target effects

P6

Sublethal effects of two reduced-risk insecticides on the predatory mite *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae)

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Abstract: The predatory mite *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) is an important biological control agent of the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), in apple orchards in Quebec, Canada. Lethal effects of reduced-risk insecticides applied to control the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) have been observed mostly on predatory larvae but also on adults. Laboratory bioassays were initiated to evaluate the sublethal effects of two reduced-risk insecticides, acetamiprid and novaluron, on some biological parameters of *N. fallacis* females and the progeny.
Females of the predatory mite were deposited on apple leaves previously placed in Petri dishes infested with two-spotted spider mites. Insecticides were sprayed to runoff with an airbrush at a reduced field rate recommended against codling moth. Total fecundity was affected by both insecticides, with 32 % less eggs laid by females treated by acetamiprid compared to novaluron. Over a 12-d period, daily fecundity was also negatively affected by the two products, but for a longer period with acetamiprid than novaluron. Pre-oviposition period was significantly delayed for acetamiprid but not for novaluron compared to the control treatment. Significant effects were observed on fertility with percentages of egg hatching near 80 % for both insecticide treatments compared to 95 % for the control. The development time of the progeny (egg to adult) of treated females were significantly higher with acetamiprid than novaluron. These insecticides also affected similarly the consumption of treated preys. Female predators consumed less than half of the preys' number compared to the control. In general, the acetamiprid has caused more sublethal effects than novaluron suggesting that the impact of the latter on the populations of *N. fallacis* in the apple orchards would be less than that caused by acetamiprid.

Key words: Integrated pest management, insecticide toxicity, contact exposure, contaminated prey, ingestion

P7

Fauna of beneficial arthropods in different managed vineyards of Zadar County (Croatia)

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Abstract: Beneficial arthropods play important role in biological control of vineyard pests. However synthetic pesticides still dominated in agricultural production. Synthetic pesticides, in particular, no selective frequently affect their population and biodiversity using negative effects. In vineyards, especially conventional often used Broad – Spectrum Insecticides and as well as a broad spectrum of fungicides. The main aim of this paper was to research the abundance and biodiversity of beneficial arthropods in three different managed vineyards (conventional, extensive and integrated). The research was carried out from May to October in 2016. Samples were collected every fifteen days by beating technique, using the entomological net. Species richness and biodiversity was calculated using Shannon Wiener Index, while Sörensen Index was used to compare similarities between localities. In total 175 arthropod individuals belonging to spiders and to insects were trapped. More individuals were presented in integrated and extensive than in conventional vineyard. The highest number of spiders was found in extensive vineyard whereas the lower in the integrated one. Between all captured insects families Chrysopidae, Coccinellidae, and Syrphidae dominated. The highest abundance respectively showed *Chrysoperla carnea* (Stephens, 1836) (25). In the conventional vineyard, *Deraeocoris schach* (Fabricius, 1781) was represented by only one specimen. The highest biodiversity index was also reported for samples in the integrated vineyard (Shannon Wiener Index - 2.161). The highest similarity showed integrated and extensive vineyard.

Key words: biodiversity, natural enemies, syntethic pesticides

P8

Ants and agriculture: a preliminary study of the ant community on annual and perennial crops

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Abstract: Ants (family Formicidae) are dominant insects of terrestrial ecosystems, whose activities are known to increase soil nutrient content (mostly nitrogen and phosphorus) and change its chemical and physical properties, including improving water infiltration due to their burrowing activities. Utilizing the ecosystem services of naturally occurring insects is an under-appreciated approach in agricultural intensification, but several studies have shown up to a 36% increase in crop yield due to the presence of ants on the studied fields. Furthermore, because some ant species are sensitive to land management, they are successfully used as indicators of soil-based ecosystem function in agricultural management throughout the world, but mostly in warmer climates. In order to investigate the ant communities associated with different agricultural practices in intensive crop production in Croatia, we sampled ants using pit-fall traps on annual and perennial crops, as well as in the natural area at the same locality. We present our preliminary findings and discuss future direction of using ants as ecological indicators and key regulators of important soil processes.

Key words: Formicidae, soil, ecological indicators, agroecosystems

P9

The efficiency of azadirachtin and organic fertilizer as environmentally friendly products in pest control

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Abstract: Neem is a long known botanical insecticide whose main active ingredient is azadirachtin. Azadirachtin acts as a regulator of growth and development, and also interferes with feeding and egg laying. The use of various organic fertilizers or reinforcements for plants can also reduce the damages on plants. We investigated the efficacy of azadirachtin (product 'NeemAzal T/S') and product based on organic nitrogen fertilizer ('Boundary SW') against western flower thrips and tobacco flea beetle adults. In laboratory trials, azadirachtin was tested in three doses (1.5 l/ha, 3.0 l/ha, 4.5 l/ha) and fertilizer in a dosage of 3.0 l/ha, in four replicates for both pests, including an untreated control. All variants included ten specimens per replication. For thrips, products were applied by foliar spraying of bean plants, whereas for flea beetles the tobacco plants were treated according to the IRAC No7 method. The test was read every 24 hours for three (thrips) or four (flea beetles) days. The number of dead beetles on insecticide and control was used to calculate the efficacy by using the Schneider-Orelli formula. Besides efficacy, for thrips the damage on leaves and the number of eggs laid were also recorded. The results showed that azadirachtin was not sufficiently effective (66.0%) against western flower thrips adults. However, after 72 hours the highest dose reduced their feeding, and accordingly, the damages on the leaves (4.0%). The same dose also prevented egg laying completely, thus interrupting the life cycle of insect pest. The organic fertilizer showed low efficacy (12.5%) on thrips, but it was effective on thrips feeding and reduced the damages on bean leaves (11.3%). For tobacco flea beetle, the highest dose of azadirachtin showed excellent efficacy (92.1%) after 96 hours. The organic fertilizer was not as effective and showed the efficacy of 59.6%. Considering these results, azadirachtin could be considered as an alternative for chemical insecticides and used for both western flower thrips and tobacco flea beetle control, whereas the organic fertilizer should be investigated further in combination with other biological or chemical products. Secondary effects of some insecticides, such as on feeding and egg laying, should be taken into account in pest control.

Key words: efficacy, *Epitrix hirtipennis*, *Frankliniella occidentalis*, neem, organic fertilizer

P10

MEDITERATRI project (Croatian Science Foundation) - understanding the effect of pesticide on non-target invertebrates through trophic interactions in Mediterranean Agriculture

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Abstract: Olives and grapes are considered to be among the most significant crops common to the Mediterranean region. Even though they are well adapted to the Mediterranean type of climate, still the events such as invasive alien species, climate changes, etc. can significantly affect these ecosystems causing ecological and economic harms. Neonicotinoids, as the most commonly used insecticides worldwide in last decade, recently have been banned in many countries due to their adverse effects on non-target organisms (insects, birds, etc.), and raised great concerns about the survival of beneficial predatory species (e.g. carabid beetles and spiders), important for pest control and essential for healthy ecosystem functioning. On the other hand, the application of Copper (Cu) has a long historical use, especially in the Mediterranean agriculture.

High biodiversity in Mediterranean biogeographical region and thus very complex food webs are far from being well explored which makes it hard to predict all possible negative effects of applied pesticides on non-target predatory fauna. Within the MEDITERATRI project we want to explore the effects of two types of management, IPM and ecological, on trophic interactions between beneficial predatory invertebrates and their prey, their diversity and field abundance, and to analyse certain traits that might be important for the survival of beneficial fauna in the field.

Field survey takes place within Mediterranean agriculture ecosystems, in vineyards and olive orchards, treated with both pesticides (thiamethoxam and Copper) and in pristine habitat. Metagenomics will be employed to reveal the complete diet of predatory invertebrates in the field, by identifying and barcoding all the relevant species in the field sites that have not already been barcoded. LC-MS/MS and ICP-MS technique will be used to quantify neonicotinoid residues and Cu concentrations in soil and animals representing different trophic guilds.

The results will significantly contribute to the risk assessment of pesticides proliferation in the ecosystem as well as the knowledge on the overall field sustainability of predatory invertebrates as a key group in integrated pest control.

Key words: beneficial fauna, biocontrol, Copper, IPM, thiamethoxam, Next Generation Sequencing, olive orchards, vineyards

P11

Toxicity assessment of the selected insecticidal products on Asian ladybeetle (*Harmonia axyridis*, Pallas 1773)

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Abstract: The Asian ladybeetle (*Harmonia axyridis*) (Pallas, 1773) is an invasive species originating in East Asia. In the area of protection of fruit crops against pests, *Harmonia axyridis* is considered to be a significant predator of aphids, psyllas and other pests. In compliance with the principles of integrated fruit production, it is necessary to decide rationally on applied pesticides with regard to the negative impact of individual products on non-target organisms, especially predators. The intention of the study was to find out whether the selected pesticides could negatively affect the birth rate of adults and the ability to develop pupae in an adult ladybird. For this purpose, 17 active substances were tested (11 insecticides and 6 fungicides). All tested active substances can be used by the growers in the Czech Republic under IPM. Seven of them can be used in the ecological system of fruit growing. The active substances thiacloprid, chlorantraniliprole, methoxyfenozide, imidacloprid, acetamiprid, pirimicarb, chlorpyrifos-methyl, spinosad, indoxacarb, flonicamid, abamectin and fertilizers of different active substances with fungicidal effects were tested. The primary parameter of evaluation was mortality. Contact efficacy of the products was evaluated in adults after 24 and 48 and 72 hours, in pupae up to 144 hours. The positive results, i.e. that the active substances are not toxic to ladybeetle (pupae and adults), have been achieved with the active substances chlorantraniliprole, methoxyfenozide, pirimicarb, spinosad, flonicamid and all fertilizers with fungicidal effects except Red Seaweed extract fermented rich in iodine (25%). Adult and pupal mortality for these active substances was between 0 and 10%, with the exception of indoxacarb and imidacloprid, which caused high mortality in adults (95% and 70%). Higher adult mortality was observed for the active substances thiacloprid (40%), abamectin (70%), acetamiprid (100%) and chlorpyrifos-methyl (100%). Low mortality in pupae was also noted after application of the active substance thiacloprid (10%). On the contrary, high mortality occurred after acetamiprid (60%), abamectin (85%) and chlorpyrifos-methyl (95%).

Key words: Asian ladybeetle, *Harmonia axyridis*, predator, mortality

P12

Pesticide residues in carabid beetles

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Abstract: Carabids are predators of insects and small invertebrates, they also feed on weed seeds but simultaneously present important food source to animals at a higher trophic level. As such, they are often used in research especially those related to agrochemicals. Intensive production with higher pesticide input has been highlighted as critical issue that led to a decrease in number of beneficial organisms. In this research, we aimed to investigate are there pesticide residues present in carabids collected in one of the most typical agroecosystem i.e. crop production area. The research was conducted in eastern Croatia on two distinct localities Lukač and Tovarnik during 2016. At each location carabid samples were collected in May, July and September on sugar beet fields grown as three different variants: (i) from untreated seed - control; (ii) from seed treated with imidacloprid; (iii) from seed treated with thiamethoxam. During vegetation all other standard agrotechnical measures were conducted as well. Samples were collected using covered pitfall traps with saline solution. Pesticide residue analysis on 300 different active ingredients was carried out by a certified laboratory using an analytical method based on the QuEChERS approach and high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) whose limit of quantification is 0.001 ppm. Data on weather conditions were also collected at closest meteorological stations. Higher catches of carabids were recorded in Lukač what is in relation to a conservation type of tillage, lower input of agrochemicals, lower temperature and more humid area which are better suited to carabid optimal development conditions. The analysis revealed that four insecticides, six fungicides and six herbicides were found in carabid samples. Insecticide fipronil was detected in Tovarnik at concentration 0.040 ppm while in Lukač we found imidacloprid in concentration of 0.027 ppm. Technically pure imidacloprid has proven to be lethal for *Poecilus cupreus* L. larvae at concentration of 0.4 mg/kg, while there is no data for other insecticide groups. Since all neonicotinoids are from now on banned form use by EU Commission Regulation from 2018, lower pressure on beneficial organisms can be expected. Among six fungicides epoxiconazole was detected at both locations with concentrations between 0.016 ppm in Tovarnik and 0.052 ppm in Lukač. According to PPDB, epoxiconazole has been shown to have moderate to high toxicity on sediment dwelling organisms and invertebrates. It is persistent in soil and has the ability of accumulation in tissues. Herbicide ethofumesate was also present at both localities, but at much higher concentrations in Tovarnik (0.281 ppm). According to the PPDB there is no recorded negative impact of ethofumesate on arthropods present in the soil. Determined residues are too low to make conclusions about their negative impact but it should be taken into account that some pesticides may bind to the body's fatty tissue. If knowing that fungicides, next to insecticides, are a group that has been shown to have a negative effect on beneficial organisms, more attention should be given to possible adverse effects and accumulation in carabid beetles.

Keywords: Carabids, residues, insecticides, fungicides, herbicides

P13

Assessment of survival, reproduction and foraging behavior in bumblebees (Bombus terrestris) by exposure to benzethonium chloride, a non-peptidyl agonist of myosuppressin

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Abstract: In this project we investigated for the potential side-effects of benzethonium chloride (Bztc) that is a non-peptidyl mimetic analogue of myosuppressin (TDVDHVFLRFamide) (Lange et al., PNAS 1995), on the buff-tailed bumblebee (*Bombus terrestris*), an important generalist pollinator of wild plants and many agricultural crops. We assessed for effects against survival, food intake, reproduction and foraging behaviour. The tests were done with queen-less bumblebee micro-colonies at standardized conditions (Mommaerts et al., ECTX 010). The bumblebee workers were exposed via the drinking sugar water, starting from Bztc at 0.1, 1, 10, 100 to 1000 µg/ml. This work on environmental risk assessment with beneficial insects as pollinators was performed in the frame of the development of neuropeptide analogues as novel insecticide agents. In addition, we did an important observation that the highest concentration of the formulation posed a repellent activity in a PER (proboscis extension reflex) assay with bumblebee workers.

P14

Laboratory assessment with bumblebees (*Bombus terrestris*) supports no sublethal effect of sulfoxaflor on foraging behavior

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Abstract: Sulfoxaflor is a recently developed systemic sulfoxamine insecticide (IRAC 4C) that is highly efficient against a wide range of pest insects. In this project, we tested if there are side-effects of sulfoxaflor on the foraging behavior of the buff-tailed or large earth bumblebee (*Bombus terrestris* L.), that is an important generalist pollinator of wild plants and many agricultural crops worldwide. To do this we made use of a laboratory bioassay with queen-less bumblebee microcolonies that has been developed before to assess for lethal and sublethal effects on foraging behavior, and clear effects were reported for the neonicotinoid insecticides imidacloprid and thiametoxam (Mommaerts et al., ECTX 2010). The workers were treated orally via the food for a period of 15 days; we supplemented sulfoxaflor in the drinking sugar water, overspanning a range of field-realistic concentrations of 1, 5 and 15 µg/ml, and scored for loss of survival. To the best of our knowledge, this is the first study evaluating the risk of this new compound on the foraging behavior of a general pollinator, and we believe these data can therefore be of use in the debate on neonicotinoid insecticides specifically and/or risk assessment of pesticides with a potential behavior effect on beneficial organisms in general.

P15

Introduction to the iPLANTA COST Action and RNAi research and development in relation to crop protection

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Abstract: In the short 20-year period since the first reports of RNA interference there has been a tremendous growth in research and associated publications. We know that RNAi is an endogenous cellular process that occurs naturally to "turn off" unwanted or harmful specific nucleic sequences, or to regulate gene expression before translation. RNAi has been reported in many organisms including fungi, animals, ciliates, and has more recently been studied in plants. Research has initially focused on using RNA to silence genes in order to study gene functions in the model species *C. elegans* (nematode) and *Drosophila* (Diptera) and in model plants such as *Arabidopsis* and tobacco. This research has contributed to revealing potential conserved target genes in other invertebrates, vertebrates, and humans that can be used for remediating disorders and controlling pest species.

RNAi functions in both plants and animals utilizing the dsRNAs as a trigger that targets homologous mRNAs for degradation or inhibition of its transcription. Consequently, post-transcriptional gene silencing has emerged as a method of choice for gene targeting in fungi, insects, bacteria and plants. Presently, there are several routes of gene silencing identified in plants, these include: post-transcriptional gene silencing (PTGS), transcriptional gene silencing (TGS), microRNA silencing (miRNA). The long dsRNA precursors, which are homologous in sequence to the target gene to be silenced, initiate the process of RNAi in the cell's cytoplasm, where they are processed into 21-24 nucleotides long siRNA molecules (siRNAs) by the RNase-III-like enzyme Dicer (DCL). These siRNAs are unwound into the passenger and guide strand; the latter is bound to the RNA-Induced Silencing Complex (RISC), to find a specific mRNA site and to cleave it. The gene is 'silenced' by mRNA destruction or by preventing its translation.

The most recent discovery in RNAi silencing is the cross–talk occurring between kingdoms. Studies carried out on plants and their fungal pathogens in the laboratory indicate that both parties can move RNAs back and forth into each other's cells. Fungal microbes utilise RNAi to enhance their spread, whereas plants seem to use these molecules to counter infection from these pathogens. Both types of organisms achieve their desired outcomes through the same molecular process of RNA interference, which disrupts gene expression by degrading target messenger RNAs.

In plants it is now recognised that they can be modified to express dsRNA which target genes in pests and pathogens parasitizing plants, as well as endogenous plant genes. One of the most common applications of RNA interference is the induction of pathogen derived

resistance (PDR) based on the expression of pathogen genetic elements (Baulcombe, 1996), which has led to various forms of plant virus resistance often based on the induction of an RNA-mediated mechanism. Currently RNAi is being studied and developed for controlling plant pests and viral, fungal and bacterial pathogens as both a pesticide and expressed in GM plants. These methods to exploit plant defence mechanisms or changing plant metabolism by RNA silencing show great potential. Gene expression in pathogens and pests can be targeted and plants modified to produce dsRNAs which trigger silencing and affect essential physiological functions in pest or disease-causing organisms. The development of transgenic plants expressing dsRNA has resulted in several commercial releases such as the virus resistant papaya and the corn rootworm resistant maize. However, many of the modes of activity of the micro- and small interfering RNAs (miRNAs, siRNAs) that mediate the silencing effect are not yet fully understood and knowledge of systemic propagation, turnover, specificity and activity of these molecules is limited. COST Action CA15223 iPLANTA is determining the most important research tasks for the development of these novel transgenic strategies.

Reviews of current knowledge on RNAi will be conducted by iPLANTA and supplemented by the reviews being conducted by EFSA and others. The first EFSA review of the molecular information on plant RNAi was published recently (Paces *et al*., 2017) and reviews related to environmental and food/feed issues are currently in progress.

In order to assess the biosafety of these new RNAi systems, information is required on a range of biosafety aspects related to the specific activity of RNA based silencing molecules in crop plants and in exposed biota. The biosafety aspects of plant RNAi used for crop protection have been considered in many studies and reviews (e.g. Zang *et al*., 2017, Limera *et al*., 2017). Currently, research regarding the presence and persistence of si- or mi-RNAs is contradictory and genomic data are limited for non-target organisms, so in addition to specificity, it is critical to consider exposure to dsRNAs in the risk equation for non-target organisms. Exposure also ties in with the immune stimulation and RNAi machinery saturation causing potential unintended effects, so they need to be addressed together. A task of iPLANTA is to conduct some state-of-the-art case study risk assessments of GM RNAi plants and then use this information to recommend data requirements for the risk assessment of these plants which can be communicated to EFSA and to regulatory authorities.

Further information on COST Action CA15223 iPLANTA is available at: www.iplanta.univpm.it and http://www.cost.eu/COST_Actions/ca/CA1522.

P16

Toxicity of insecticides used in cotton crop to adults of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) through different routes of exposure

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Abstract: For the biological control of pests in the cotton crop in association with chemical control it is important that the insecticides are selective to the natural enemies. On its context, the objective of this work was to evaluate the toxicity of the insecticides teflubenzurom, thiodicarb, chlorfenapyr, flupyradifurone and methomyl in their higher dosages on *Trichogramma pretiosum* using different routes of exposure. Topical exposure, ingestion and contact bioassays were conducted in the laboratory with adults of *T. pretiosum*. The residual effect of insecticides considered to be moderately harmful and harmful to the parasitoid in the laboratory contact bioassay was also evaluated under semi-field conditions (greenhouse), according to IOBC. Methomyl and thiodicarb were the most toxic insecticides for adults of *T. pretiosum*. Teflubenzurom was slightly toxic when sprayed directly on wasps or when parasitoids kept contact with their wastes on inert surfaces; however, caused a reduction in parasitism (F0) when ingested. Flupyradifurone reduced the number of parasitized eggs in the contact bioassay and was classified as moderately harmful, methomyl and thiodicarb were harmful insecticide. In the residual persistence bioassay, chlorfenapyr, methomyl and thiodicarb were classified as persistent (> 30 days of reduction of the beneficial capacity of the parasitoid). Teflubenzurom and flupyradifurone presented low toxicity to *T. pretiosum* adults and should be preferred in integrated pest management programs in cotton for the maintenance of this natural enemy. The other insecticides must be evaluated under field conditions for the confirmation of the toxicity to that parasitoid.

Keywords: *Gossypium hirsutum*, pests, parasitoid, physiological selectivity, ecotoxicology

P17

Effect of insecticides on *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) walking behavior

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Abstract: Among the beneficial insects present in the corn crop, *Doru luteipes* (Scudder) stands out as an important predator of *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). However, the chemical control of this insect is quite common and can cause negative effects to the predator. Thus, this study was developed to evaluate the effects of flubendiamide (Belt® - 125 mL/ha) and chlorantraniliprole (Premio® - 125 mL/ha) on *D. luteipes* walking behavior. The products were mixed in water:acetone (1:1), from which 1 µl was withdrawn and topically applied to the thorax of each adult insect. After application, the insects were individualized in Petri dishes, containing water source and food *ad libitum*. After 24 hours of exposure, each surviving insect was submitted to a ten-minute walk test in Petri dishes using a video camera (Logitech® c270 HD 720p) connected to a computer to capture the images, which were analyzed through the video tracking program EthoWatcher®. The bioassay was conducted in an air-conditioned room at 25±2°C, RH of 70±10% and photophase of 12 hours. The experimental design was completely randomized, with three treatments and four replicates, each consisting of three adult insects. In the treatment with chlorantraniliprole, the distance traveled (7.02 cm) and the walking velocity (0.12 cm/min) were lower compared to flubendiamide and control treatments (12.63 cm and 0.21 cm/min). It was also observed that males, in general, presented higher speed and walking distance than females. The results indicate that adults of *D. luteipes* contaminated with chlorantraniliprole may present less foraging and consequently affect their performance as a population regulator of *S. frugiperda* in the field.

Keywords: *Zea mays*, earwig, physiological seletivity

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