ABSTRACTS:

Insect Pest Management:

Poster Presentations

In programme order

Poster Session 4

TV PheroLure®: The influence of a semiochemical lure on the volatile profile of a commercial tomato field

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Pheromone-based or semiochemical lures for insect detection and monitoring in agriculture is common practice. Many countries exempt these devices from regulatory requirements, however, not South Africa. The question arises whether the pheromone/semiochemical lures influence the naturally occurring compounds significantly, to justify concern for human toxicity and ecotoxicity. T.V. PheroLure® is a novel five-component lure developed by Insect Science (Pty) Ltd. used for monitoring African bollworm, *Helicoverpa armigera* (an important insect pest on tomatoes). T.V. PheroLure® is a volatile organic compound (VOC) blend impregnated in a polyethylene bulb. The influence of T.V. PheroLure® on the volatile profile of a tomato field was evaluated in a commercial growing area of South Africa. Tomato VOCs were collected before, during, and after the application of six T.V. PheroLures® in yellow bucket funnel traps randomly distributed over one hectare. VOCs were collected from planting until harvest (22 weeks) at five randomly selected sites at different heights (0 cm, 30 cm and 60 cm above ground level). Collection also took place in adjacent tomato fields where no T.V. PheroLure® was applied. The constituents of T.V. PheroLure® had no significant influence on the naturally occurring VOCs observed in the tomato field, irrespective of sampling height. Suggesting that the concern for toxicity and ecotoxicity is unjustified when using semiochemical devices for monitoring purposes. The natural physiology of the plant, rather than T.V. PheroLure®, influenced the VOCs observed in a tomato field.

Keywords: Insect monitoring; African bollworm; South Africa; Toxicity; Volatile organic compound (VOC)

Oviposition cues for the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae)

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The Asian citrus psyllid (ACP) is the vector of Huanglongbing, the most destructive citrus disease. During host plant search, ACP uses visual, odor and contact cues from flushing shoots. A preference greenhouse experiment performed using 6 citrus species: Murcott and Clemenules Tangerine; Valencia, Navelina and Lanelate Oranges, and Lisbon Lemon shown that ACP prefers to oviposit on tangerines, being the number of eggs at least two-fold than on the other varieties (GLMM, p<0,05, N=16). Considering the number and length of shoots by variety, tangerines developed more (29±7 vs. 17±9, $p < 0.05$) and shorter shoots (3.3±1.5 vs 4.2±1.8cm, p=0.05). In turn, the oviposition preference (N°eggs/species) at the end of the bioassay positively correlates to the number of shoots (R^2=0.30, p=0.003). Searching for biomarkers that may serve as kairomones for ACP females, the volatile organic compounds (VOCs) and the epicuticular waxes from shoots of all varieties (N=8/species) were analyzed (GCMS). All data were processed using PARADISe software. Eighty four peaks were detected in VOCs profiles and 140 in wax profiles. Univariate analyses allowed to identify, among other terpenes, that limonene was in higher amounts and caryophyllene in lower amounts in tangerine VOCs compared to all other varieties. Besides tangerine waxes exhibited a lower amount of a still unidentified fatty alcohol and a higher amount of an unidentified diterpene (p<0.05 in all cases). These results suggest that under experimental conditions, the ACP could use a combination of biological (number of suitable shoots for oviposition) and chemical cues to choose its oviposition plants.

Keywords: Epicuticular waxes; Kairomones; Metabolomics; Preference; Volatile organic compounds VOCs.

Development of Odorant Binding Protein based Biosensor for early detection of Red Palm Weevil

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The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* is one of the most invasive and globally important quarantine pest of palm trees. Early detection of such infestations is highly important for commercial crops but no such systems yet exist. Male produced aggregation pheromones may be involved in coordination of mass attacks on trees. Odorant Binding Proteins (OBPs) are small soluble proteins present in the olfactory systems of vertebrates and insects having affinity to various volatile organic molecules. Because their high conformational stability, OBPs are attractive biorecognition elements to create sensors for different applications. The pheromone-specific OBPs (*RferOBP1768* and *RferOBP23*) were recently identified from the Red Palm Weevil. These were incorporated onto Quartz Crystal Microbalances (QCMs) to produce sensors for detection of the aggregation pheromones, 4-methyl-5-nonanol (ferrugineol) and 4-methyl-5-nonanone (ferruginone), together with a kairomone, ethyl acetate to be used for early detection of RPW infestation in date palms. The tertiary structures of the OBPs were modelled to study the ligand binding sites *in silico* and the proteins were expressed and purified. The expressed OBPs were characterized by ligand binding studies in solution. The proteins were immobilised on QCMs using a self-assembled monolayer approach. The resulting sensors were exposed to pulses of vapours from target analytes and were able to selectively and sensitively recognise in vapour phase the two aggregation pheromones ferrugineol and ferruginone at very low concentrations (ppb levels). The biosensors remain stable and respond to analyte vapours for up to one year.

Keywords: Red Palm Weevil; Biosensor; Odorant Binding Protein; pheromone detection; aggregation pheromone

A novel olfactory protein-based concept for the Red Palm Weevil early detection and control

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The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) is reported in almost all the dominant palm tree-growing countries worldwide. RPW recently attained quarantine category – 1 pest status, which, if not eradicated, would cause significant damage to the palm trees. Here we propose a novel olfactory concept that, combined with functional genomics approaches, will help long-term pest management solutions for the RPW control. Recently, we reported the molecular basis of pheromone, (4*RS*,5*RS*)-4-methylnonan-5-ol (ferrugineol), detection in RPW, through functional characterization of ferrugineol-specific odorant-binding proteins – OBP (*RferOBP1768*). Here, we complete our view of ferrugineol detection by the identification of the corresponding odorant receptor (*RferOR1*) *via* RNAi and heterologous expression. These data are being used to develop ferrugineol-specific OBP/OR- based biosensors. Through an international network of interdisciplinary research, we aim at immobilizing *RferOBP1768* and/or *RferOR1* on Quartz Crystal Microbalance biosensors for the precise detection of the palm weevil pheromone, with the long-term practical application for early detection of RPW adults in the fields.

Keywords: Red palm weevil, olfactory proteins, odorant-binding protein, odorant receptor, biosensor.

Trialeurodes vaporariorum settlement preference and its relationship with the volatiles emitted by the tomato plant

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In the complexity of ecosystems, herbivore insects need to select the right host plant to be able to develop and accomplish its life cycle. This choice is often guided, among other cues, by plant volatiles. *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), a polyphagous and cosmopolitan pest, is one of the main pests of tomato (*Solanum lycopersicum*, Solanaceae) in Uruguay. To characterize the interaction mediated by plant volatiles between tomato and this whitefly, fully grown tomato plants were damaged with 75 adults of *T. vaporarorium* for 24 h. These damaged plants were then offered together with an undamaged plant to another set of 75 whiteflies (N = 11). Settlement preference was measured at 24, 48 and 72 h. *T. vaporarorium* always preferred settling on damaged plants (day 1: 44 ± 5 vs. 17 ± 2 on damaged vs undamaged plants; day 2: 46 ± 6 vs 16 ± 3; and day 3: 38 ± 6 vs. 13 ± 2. p=0.006, p=0.001, p=0.021 respectively, Wilcoxon tests). Headspace collection was also performed for healthy and damaged plants during 72 h. Comparison of 83 GCMS peaks in a fold change (double or more) analysis allowed the detection of 16 compounds that increased in damaged plants, and 2 that decreased ($p < 0.05$). Future studies will focus on characterize whether the whitefly settling behavior correlates with the change of these compounds in damaged plants and whether other cues are also used.

Keywords: herbivore; whitefly; VOCs; HIVPs; plague

The major pheromone component identity of the Pine Emperor moth, Nudaurelia clarki (Lepidoptera: Saturniidae), is confirmed

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Nudaurelia spp. (Saturniidae) are sporadic pine plantation pests in South Africa. The development and implementation of pheromone-based management tactics in South Africa has been limited by taxonomic uncertainty with this group. We compared *Cytochrome oxidase subunit I* (COI) gene sequences from historical and contemporary samples of moths from the KwaZulu-Natal (KZN), Western Cape and Mpumalanga regions that have previously been reported as *Nudaurelia clarki* and *N. cytherea* in literature. Female pheromone gland extracts were also analyzed with gas-chromatography electroantennographic detection (GC-EAD) and gas-chromatography-mass spectrometry (GC-MS). *COI* gene barcoding sequences were identical for sampled moths from all considered regions suggesting that these populations may be the same species. The samples collected for this study were considered to belong to *N. clarki*. Male *N. clarki* antennae respond to two compounds in female extracts. One of these compounds was confirmed with a synthetic standard to be (*Z*)-dec-5-en-1-yl-3-methylbutanoate, the sex pheromone previously identified from *N. cytherea*. The identity of the second compound could not be confirmed. Both male and female antennae responded to four structurally related compounds in the synthetic pheromone standard. Custom-made traps fitted with polydimethylsiloxane and polyisoprene lures loaded with synthetic (*Z*)-dec-5-en-1-yl-3-methylbutanoate confirmed attraction of *N. clarki* males to the pheromone in field trials. The shared pheromone chemistry between populations previously considered as *N. cytherea* and *N. clarki* further promotes the hypothesis that they are the same species.

The hydrocarbon footprints profiles of three predaceous Coccinellidae (Coleoptera) are species and gender-specific

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Eriopis connexa (Germar) and *Tenuisvalvae notata* (Mulsant) are coccinellids native to South America, whereas *Cryptolaemus montrouzieri* Mulsant is an exotic species, introduced in the 90s. These species are important biological control agents of aphids and mealybugs, respectively. However, when they occur simultaneously in an area, they may compete for food niche. Footprints of these species can influence intraspecific and interspecific recognition, affecting their behavior. Thus, the objective of this study was to evaluate the chemical profiles of footprints of these three coccinellid species. The footprints were extracted from glass Petri dishes with hexane, identified and quantified by GC-FID and GC_MS. Fifty-two different aliphatic hydrocarbons have been identified in all tree species. In *E. connexa* footprints, twenty-two saturated hydrocarbons were identified, most of them methyl-branched hydrocarbons (ranging C_{23} to C_{31}). In *T. notata* footprints, seventeen hydrocarbons were identified (ranging C_{25} to C_{35}), ten unsaturated. Finally, in *C. montrouzieri* there were twenty-three hydrocarbons (ranging C_{21} to C_{31}), twelve of them unsaturated. Two linear hydrocarbons, pentacosane and heptacosane, were common to all species, but their quantity was species-specific. In addition to the differences between species, there was a qualitative and quantitative difference in hydrocarbons between the genders. The differences on the chemical profile of the footprint hydrocarbons can indicate that the behavior recognition may be related to specific stimuli. These results can contribute to the adequate management of the studied coccinellids, aiming the control of pests that damage production.

Keywords: behavior; biological control; chemical communication; ladybugs; semiochemicals.

How does the combined use of humic substances and beneficial bacterial affect coffee plant resistance to mealybugs?

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Humic substances (HS) and beneficial microorganisms have been shown to promote greater plant growth and ameliorate abiotic and biotic stresses. However, little is known about effects of the combined use of these two bioestimulants on plant resistance to insects. Here, we investigated how the combined and single use of HS and the plant growth-promoting bacterium (PGPB) *Enterobacter tabaci* influenced resistance of *Coffea arabica* to the white mealybug *Planococcus minor*. We evaluated resistance by assessing the mealybug's host selection and performance assays and by quantifying total phenols of HS-treated, PGPB-treated, HS+PGPB-treated and untreated coffee plants as a putative direct defense. Treatment with any of the biostimulants promoted greater shoot growth relative to that of untreated coffee plants. In four-choice assays, mealybugs were found in lower numbers on HS-treated, PGPB-treated and HS+PGPB-treated plants compared to the control, but the lowest infestation was on HS-treated plants. The mealybug performed poorly on HS-treated and HS+PGPB-treated compared to untreated control. Despite the greater resistance in some of the bioestimulant treatments, the quantification of total phenols was similar among the treatments, suggesting that their levels do not play a role in coffee resistance against the white mealybug. In conclusion, our results showed that treatment with HS promotes increased resistance of coffee plants to the white mealybug, while the PGPB showed a discrete positive effect on resistance. The combined used of the bioestimulants did not yield a synergistic nor an antagonistic effect on coffee resistance against the mealybug.

Keywords: bioestimulants; herbivore performance; host selection; phenols; plant growth promoting bacteria.

Host selection responses of Asian citrus psyllid (*Diaphorina citri*) to salinity stressed and unstressed citrus seedlings

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The Asian citrus psyllid (ACP), *Diaphorina citri*, vectors *Candidatus* Liberibacter asiaticus and *Ca.* Liberibacter americanus, the putative causal agents of Huanglongbing (HLB), the most destructive citrus disease in the world. Currently no therapies exist for treating infected trees, which typically become unproductive and die. Growers rely entirely on insecticides to control ACP, and sustainable control methods are needed. ACP incidence is dependent on the presence of shoots, where it reproduces and develops. Because salinity stress affects shoot physiology, we hypothesized that that moderate salinity stress could interfere with host selection by ACP. Here, we evaluated ACP host preference when presented with a pair of Rangpur lime (cv. 'Cravo Santa Cruz') seedlings that had been exposed to either low saline (1.7 dS m^{-1}) solution or highly saline (10 dS m^{-1}) solution. At 15 and 20 days following the imposition of salinity stress, pairs of unstressed and stressed seedlings were placed inside screened cages (45 cm length, 42 cm width, 42 cm height) containing 30 adult ACP. After 48 h, the number of ACP on each seedling was evaluated. Significantly higher proportions of ACP settled in the unstressed seedlings than in the salinity-stressed seedlings. Key physiological responses, such as net photosynthesis, transpiration and osmotic potential, were significantly lower in salinity-stressed seedlings. These physiological changes may have influenced ACP host selection behavior. Strategies for the insect management can be developed based on this knowledge, especially in semi-arid and arid areas where citrus is irrigated with water that has low to moderate salinity.

Keywords: abiotic stress; Asian citrus psyllid; biotic stress; greening; plant-insect interaction

Trapping of *Retrachydes thoracicus thoracicus* and other South American cerambycid beetles in combined pheromone and plant kairomone traps

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Sex-aggregation pheromones in longhorn beetles show remarkable chemical parsimony. Similar structural motifs such as α-hydroxyketones have been found in numerous species, sometimes working in combination with plant volatile kairomones. *Retrachydes thoracicus thoracicus* (Olivier, 1790) is a polyphagous South American cerambycine beetle with unknown pheromone chemistry. Over the past 4 years, we have been conducting field studies with cerambycid pheromones in citrus orchards located in southern Uruguay, with significant incidental captures of *R. thoracicus thoracicus* in cross-vane traps lured with racemic 3-hydroxy-2-hexanone. In the 2020-21 summer season, an experiment was performed to compare the attraction of lures composed of neat 3-hydroxy-2-hexanone, 3-hydroxy-2-hexanone plus lemon essential oil, and 3-hydroxy-2-hexanone plus ethanol. An absolute control with empty lures was also performed. After eight weeks of captures, the results showed a remarkable increase in *R. thoracicus thoracicus* trap captures when 3-hydroxy-2-hexanone was added with ethanol (43 ± 6 insects) compared to 3-hydroxy-2-hexanone plus citrus volatiles (0.6 ± 1.1) and 3-hydroxy-2-hexanone alone (1 ± 1). Consistently, more females (132) than males (3) where caught. Smaller numbers of eight other native cerambycid species were also caught in pheromone-lured traps, suggesting that they either produce 3-hydroxy-2-hexanone for intraspecific communication, or they "eavesdrop" on the pheromone communication system of other guild members, as has been reported for other species. The strong synergistic effect of ethanol in the attractiveness of 3-hydroxy-2-hexanone is likely explained by its kairomonal role as a cue for plant stress or ripeness.

Keywords: Longhorn beetles; Cerambycinae; kairomone-pheromone synergism; 3-hydroxy-2-hexanone; ethanol

Behavioral responses to specific preys by predator coccinellids

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Coccinellids are important biological control agents, and despite that some species have generalist habits, food specialization occur within some tribes such as Scymnini. Also, prey preference or pre-imaginal conditioning may be accentuated when coccinellids are subjected to mass rearing. Then, predator-prey interaction could be a conditioned response to stimuli of the prey in which predator was reared. To assess the impact of the pre-imaginal experiences, the coccinellids *Cryptolaemus montrouzieri* and *Tenuisvalvae notata* were fed either *Ferrisia dasylirii* or *Planococcus citri*, respectively, for at least eight generations. Moreover, we measured the behavioral responses of the predators to partially treated arenas with the volatiles and trail produced by preys, regarding: i) residence time, ii) walking distance, and iii) walking speed. Finally, we investigated feeding preference in laboratory and semi-field conditions, offering both types of prey in Petri dishes, and directly on cotton plants, respectively. Coccinellids were not conditioned by specific rearing preys, as both species responded to the volatiles associated with either prey. Also, there was no difference in their responses between the control area and the trail left by prey, suggesting that trails are not enough to trigger predator response. Finally, both predator species consumed more *P. citri,* regardless of the prey they were reared. Therefore, *C. montrouzieri* and *T. notata* respond to prey's cues regardless of the prey species in which they were reared in laboratory conditions, without pre-imaginal conditioning. These results suggest that both predators are effective in the biological control of different mealybug species.

Keywords: coccidophagous predator; food specificity; massive rearing; pre-imaginal condition; prey recognition

Plants detect and respond to the presence of the third trophic level

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Interactions between plants, herbivores and herbivore natural enemies, described as tritrophic interactions, shape ecological processes in (agro)ecosystems. Recently, evidence has accumulated that plants may directly perceive and respond to the presence of herbivore natural enemies. However, this plant response and impact on the tritrophic system remain overlooked. To address this gap of knowledge, we used a tritrophic system involving maize plants (*Zea mays*), the banded cucumber beetle herbivore (*Diabrotica balteata*), and entomopathogenic nematodes (*Heterorhabditis bacteriophora*). We report a comprehensive depiction of the maize metabolomic response to entomopathogenic nematodes. We further highlight the specificity of this response and external factors that modulate it. Finally, we explore how the plant response feeds back into tritrophic interactions by showing the plant, herbivore and nematode performance.

Overall, we argue that the plant response to the presence of natural enemies may be a crucial, but underestimated, process shaping tritrophic interactions in nature, and biological control success in agriculture.

Keywords: Tritrophic interactions; Belowground interactions; Soil ecology; Entomopathogenic nematodes; Plant defenses: Root herbivory

Controlling insect pests in rice by manipulating defense responses using chemical elicitors

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Upon infestation by herbivores, plants perceive herbivore-associated molecular patterns and damage-associated molecular patterns and produce defence responses, including the release of herbivore-induced volatiles and the accumulation of non-volatile defensive compounds, by activating a defense-related signaling network. This network mainly consists of mitogen-activated protein kinase cascades and pathways mediated by jasmonic acid, salicylic acid and ethylene. The defence responses have been shown in the lab and field to reduce the fitness of herbivores directly and indirectly by attracting natural enemies of herbivores. Our previous studies with rice have found that the manipulation of defence responses, by genetic modification or application of synthetic chemical elicitors, has great potential for the control of pest populations. Here, we will show our recent findings from a novel synthetic chemical elicitor WJ-72. We found that exogenous application of WJ-72 enhances the resistance of rice and other major cereals such as wheat and barley to piercing-sucking insect pests by inducing a novel resistance mechanism. The increased herbivore resistance reduces the population of herbivores and enhances crop yield in the field.

Key Words: defensive compounds; ethylene; jasmonic acid; salicylic acid; tritrophic interaction

Behavioral response of *Sirex noctilio* towards its symbiotic fungus *Amylostereym areolatum* grown on different substrates

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The woodwasp, *Sirex noctilio* (Fabricius) (Hymenoptera: Siricidae) is an invasive species that has invaded most of the regions of the world with *Pinus spp*. Since its establishment in Argentina in the 1980s, the wasp has become one of the main insect pests affecting the forest industry. To date, an environmentally sound species-specific monitoring and control tool based on semiochemicals is lacking for this pest. In this sense, a species-specific monitoring method requires, as a basis, knowledge on the sensory ecology of the species, specifically in terms of the chemical volatiles involved in eliciting attractive responses. For instance, previous studies carried out in our laboratory have shown a strong attraction of *S. noctilio* females to the obligate symbiotic fungus *Amylostereum areolatum*.

In this context, the objective of my study was to evaluate the behavioral response of *S. noctilio* females to different volatile stimuli, focusing on those elicited by the fungus *A. areolatum* grown on different substrates (artificial agar-based culture medium or two of the pine species most widely cultivated in Patagonia: *Pinus contorta* and *Pinus Ponderosa*). Through 4-way olfactometer bioassays, I was able to determine that there is a clear hierarchy in relation to the preferences of the females towards the different stimuli evaluated. Semiochemicals emitted by the fungus cultivated in *Pinus contorta* were the most attractive. The results are discussed in the context of possible species-specific semiochemical-based monitoring and control tools and the directions of proposed future research.

Keywords: pests, semiochemicals, monitoring, control, integrated management.