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Abstract Book

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Effects of thermal history on *Bactrocera* and *Ceratitis* pests: Who flies better?

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Introduction: The fitness traits expressed by insects are strongly influenced by the thermal environment. Furthermore, it is not only the current ambient temperature that is important, but also temperatures experienced in the past (i.e., thermal history). Thermal history may induce phenotypic plasticity that may benefit or be detrimental to performance and fitness. One type of plastic response triggered by thermal history and known to alter the phenotype is acclimation. In various insect species, acclimation affects traits such as lifespan, heat or cold resistance, or flight performance. In pest tephritids, it is particularly important to understand how thermal history may affect flight performance. This is because flight is linked to invasive potential and underpins the success of pest management tactics such as the sterile insect technique.
Methods: We investigated how thermal history affects the dispersal ability of three major pests, *Bactrocera dorsalis* (Hendel), *Bactrocera zonata* (Saunders) and *Ceratitis capitata* (Wiedemann), to better understand their invasion success. To do so, we tested potential dispersal ability of both sexes of each species after acclimation at either 20, 25 or 30°C. In the laboratory, we recorded tethered flight for 2 hours using flight mills at 25°C, and related wing morphology of flies that flew the most or least. We also inferred movement of *B. dorsalis* in the field over several weeks using a mark release-recapture method in agricultural environments with varying climatic conditions.
Results: In the laboratory, *B. dorsalis* was the species that covered the most distance, and *Bactrocera* species are faster than *C. capitata*. We observed a more fractioned flying pattern in *C. capitata*, resting periods being more frequent. Overall, flies acclimated at 20°C covered shorter distances as they spend less time flying. Supporting this observation, the overall proportion of *B. dorsalis* acclimated at 20°C recaptured in the field was lower than in the other groups. Although the number of captures was temperature dependent, there was no clear pattern linking acclimation temperature to weather conditions.
Conclusions: This work provides a direct inter-specific comparison of the dispersal ability between pest fruit flies that originate from different regions of the world. This is usually not feasible due to restrictions preventing researchers from moving them into novel environments to study their dispersal abilities. We showed that *Bactrocera* species are great dispersers, and this regardless of thermal history. However, our results suggest a cool thermal history within the adult stage may reduce dispersal ability. These results are important to predict movement in these pests with regards to climate change, and may also contribute to improve success of the sterile males.

Keywords: dispersal, acclimation, tethered flight, mark-release-recapture

Environmental drivers of fruit fly phenology across climate zones

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Background: Understanding proximate drivers of species phenology is critical for their timely management and predictive modelling. Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) is a major Australian horticultural pest, but little is known about drivers of its phenology and models calibrated on data from one climate zone are not easily transferable to other parts of its range.
Methods: Here, we use a continental scale data from 2780 traps sampled over 20 years and covering a range from equatorial to temperate climate zones to document intra-specific variation in phenological sensitivity of *B. tryoni* to climate (temperature, rainfall, relative humidity, radiation). We use a sliding time window analysis, a regression-based approach which identifies the critical time window in which a climate variable explains the most variance in phenology. We further analysed changes in population short-term (within year) and long-term (among-years) abundances in relation to climate zone, habitat type, Southern Oscillation Index, distance from residential area and water bodies.
Results: We show that the identified critical time window of phenological sensitivity to climate variables was not the same for all climate zones and for all tested climate variables, but often falls within winter/dry season. This time lag between identified climate windows and population spring increase suggests that seasonal changes in population abundances are at least partly driven by climate affecting 'overwintering' adults and likely used as an environmental cue that drives species phenology.
Conclusion: Although establishing causation between an environmental cue and phenology requires experimentation, our findings suggests that climate predictors that fall outside growing season should be considered to better understand the effects of climate on dynamics on fruit fly populations.

Keywords: Queensland fruit fly, *Bactrocera tryoni*, abundance, pest, population dynamics

Session 1: Biology, Ecology, Physiology and Behavior, November 14, 2022, 11:00 - 13:05

Gut bacterial diversity and biological traits of *Bactrocera dorsalis* and *Zeugodacus cucurbitae* (Diptera:Tephritidae) under diet and temperature stress

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Background: The gut microbiota possesses diverse functions, which can positively or negatively influence the fitness of the insect host and affect the capability of showing plastic responses to environmental changes. Invasive insects often cause serious agriculture damage following their introduction into a new area, and recent studies have highlighted the key role of symbiotic communities on biological invasion. In this study, we determine the species diversity and richness of the gut microbiota in two invasive fruit fly species, *Zeugodacus cucurbitae* and *Bactrocera dorsalis* (Diptera: Tephritidae) in la Réunion. The potential role of the gut symbiont community in shaping the ability of hosts to tolerate thermic and starvation stress was also assessed.

Methods: First, we compared cold and heat tolerance of axenic (diet with antibiotic) and non-axenic tephritid fruit flies under three adult diet treatments: sucrose only (20%), yeast hydrolysate only (20%), or sucrose and yeast hydrolysate at 3 :1 ratio. For this purpose, three fitness traits (longevity, survival and female's fecundity) were assessed for each symbiont, and diet treatment combination during 60 days, and this at 10, 25, and 35°C. Then, the taxonomic characterization of bacterial community associated with axenic and non-axenic *Z. cucurbitae* and *B. dorsalis* females, reared under these stressful situations, was investigated based on a next generation sequencing (NGS) approach, using 16S ribosomal RNA (rRNA) gene amplicons.

Results: Non-axenic flies showed a significantly higher survival and higher fecundity than axenic ones at different tested temperatures. Antibiotic and diet treatments significantly affected cold and heat survival of *B. dorsalis* and *Z. cucurbitae*. Symbiont species diversity and richness were obtained and described according to each above condition tested.

Conclusion: Understanding the role of gut symbionts in insect starvation biology under thermic stress can ameliorate the prediction of the consequences of climate change on biological invasion.

Keywords: Gut-symbionts, NGS, Fitness, Temperature, Tephritidae, plasticity

Intraspecific competition by larval aggression in the olive fly, *Bactrocera oleae*

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Background: Inter and intra-specific competition in fruit flies revolve largely around the host fruit – a main resource for development and reproduction. On the host, females exclude competitors from oviposition sites by pheromones or aggressive gestures. Similarly, larvae compete inside the host while striving to secure optimal nourishment. For the most, larvae are considered to engage in scramble competition where fitness is ultimately decreased when the fruit is populated over its carrying capacity. Active measures taken to supress competing counterparts (i.e. interference competition) have been suggested for the larvae of some species but to our knowledge not identified to date.
Olive fly larvae (*Bactrocera oleae*) usually develop in solitude but occurrences of multiple infestations are not uncommon. We observed that larvae inhabiting the same fruit frequently carry multiple melanotic marks, typical of healed injuries. We hypothesized that such markings result from aggressive interactions between conspecifics (i.e. biting), and that the occurrence of aggression would be significantly affected by competition intensity. Herein, we quantify larval developmental parameters and the incidence of markings according to larval density in the fruit. Our results suggest that aggression is a major component of interference competition in olive fly larvae.

Methods: We tested our hypotheses using fruit of two olive cultivars where multiple eggs / fruit have been deposited. Weight, development time and the number of injuries sustained by each larvae that had successfully developed were subsequently quantified and compared according to larval density in the fruit.

Results: We were able to simulate typical marks by intentionally wounding larvae, thus validating their source as injuries. Regardless of fruit cultivar, the mean weight and weight gain rate of larvae significantly decreased as larval density in the fruit increased, indicating intense competition between individuals inhabiting the same fruit. The mean number of wounds per larva significantly and positively correlated with larval density in the fruit, supporting our assumption of direct aggression by conspecifics. Similar experiments conducted with *Bactrocera zonata*, whose larvae develop gregariously showed no signs of such interactions.

Conclusion: olive fly larvae inhabiting the same fruit engage in intense competition. Individuals interact not just through consuming a shared host but also by direct interference manifested as aggression (biting). Such attacks may be fatal and extend to cannibalism (personal observations). We suggest that aggression may be common to other monophagous tephritids inhabiting small hosts, and is probably restrained in polyphagous species whose larvae develop gregariously. We assume that the intractable nutritional environment in olives contributed to intensifying such behaviours in olive flies.

Keywords: intraspecific competition, larval aggression, *Bactrocera oleae*

Overwintering Mechanism of Mediterranean Fruit Fly in Western Australia

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Background: Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) is found from south-west Western Australia (WA) which is characterised by temperate climates to semi-arid desert at the northernmost part of its range. To manage medfly, growers apply insecticides as cover sprays to target eggs and larvae in developing fruits, or in bait sprays laced with protein to control adults. However, these methods are often ineffective due to poor grower understanding of medfly biology and ecology. Stonefruits grown in temperate climates in WA are particularly susceptible to medfly early in the season, with spring populations the result of overwintering life stages and adults. To improve grower understanding of medfly biology, we carried out a series of experiments to demonstrate how medfly survives winter.

Methods: The ability of medfly to overwinter in immature (egg, larva, pupa) and adult stages was tested in a series of lab and field experiments at two locations in the Perth Hills. Experiments included: (1) how they overwinter as adults (field cages), (2) as eggs and larvae in fruits such as apples (field cages, lab trials), (3) as pupae in the ground (field cages/tubes). These experiments were supported by day-degree modelling which was also used to calculate emergence dates for the spring population. We also determined whether female medfly would oviposit in winter when offered fruit and determined the distribution and abundance of wild male and female medfly over 24 months.

Results: Field experiments showed that immature stages in apples (eggs/first instar) and soil (pupae) remained viable in winter, emerging as adults at the onset of warmer weather in spring. In field cages, adults survived 72–110 days, with females laying viable eggs when offered citrus fruit. However, only 1–6% eggs survived to emerge as adults. Adults survived longer in field cages when they were caged with citrus.

Conclusion: Our results support previous research that medfly overwinters in all life stages in temperate climates WA. Overwintering adults, eggs laid in winter fruit such as apples and citrus and/or pupae in the soil are sources of new medfly populations in spring. Trapping data showed that medfly adults were present year-round with captures very low in winter to early spring. We recommend that control strategies be deployed in autumn to reduce the overwintering adult population.

Key Words: Mediterranean fruit fly, overwintering, development, lifetable

Session 1: Biology, Ecology, Physiology and Behavior, November 14, 2022, 11:00 - 13:05

Polyandry is an important reproductive trait of the invasive *Drosophila suzukii*

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Background: The spotted wing drosophila, *Drosophila suzukii* Matsumura, is a pest of soft-skinned fruit native to Eastern Asia which, since 2008, has become a world-wide pest in many Asian, American and European countries. This rapid global spread is supported by several adaptive life history traits such as a high degree of polyphagy. Very important is its ability to overwinter as "reproductively quiescent" long-lived melanized winter morphs characterized by a lack of ovary development during winter and the resumption of ovary development in spring. *Drosophila suzukii* flies possess a high reproductive rate due to a long adult lifespan and high female fecundity. To understand the population dynamics of this fly and develop control management strategies, it is important to evaluate factors underlying such a high reproductive rate. An open question is whether polyandry is a common behaviour in the wild and if so, how does the female use the sperm from multiple matings?
Methods: Females were collected from a North Italian population at the peak of its exponential growth. Paternity analyses were accomplished using SSR informative markers to determine the maternal and offspring genotypes. The remating frequencies were estimarted with the programs GERUD1.0 and GerudSim2. In addition, a Bayesian method was used to estimate parameters of multiple mating and sperm displacement.
Results: A high remating frequency was detected among the wild females of the considered population. Indeed, 83% of the tested families had been sired by more than one male, from a minimum of 2 to a maximum of 5, with an average of 3.25 fathers. Bayesian simulations strongly indicated that females from this population do not exhibit sperm displacement, β, which was estimated to be 0.504 (95% confidence level = 8.502E-05). Thus females use spermatozoa from all their male partners for siring their progeny.
Conclusion: Remating is a common event in the wild and, what is more, females of *D. suzukii*, unlike *D. melanogaster*, store sperm from different males and then use the sperm for fertilization. These are biologically relevant data for interpreting the high reproductive biology of this drosophila species. Remating influences the effective population size and may constitute a critical factor in determining the success of control methods including the SIT.

Keywords: *Drosophila suzukii*, invasive species, polyandry, sperm use

Session 2: Taxonomy and Morphology, November 14, 2022, 14:30 - 16:30

Appearance is all about diet: big-eyed generalists and big-nosed Cucurbitaceae specialists in Tephritidae

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Background: Insects rely on multiple sensory cues to initiate the many behavioural sequences necessary to complete their life cycles. Both visual and olfactory modalities are involved in adult's search for nutrients, mates or oviposition sites. Yet, recent studies in *Drosophila* revealed a developmental trade-off between sizes and functions of the head sensory organs. As a consequence each ecological niche should promote a given sensory balance. Tephritidae is an ideal insect family to challenge this hypothesis. It includes many species with similar life cycles, diverging mostly in their host range, which are bound to exhibit different combinations of visual and olfactory information.

Methods: We measured the dimensions of the following organs in Tephritidae individuals: the left hind tibia length as a proxy of body size, the width/length of the eye, and the surface of the funiculus covered by the olfactory epithelium. The study included two datasets. Firstly, a digital collection of 229 specimens from the RMCA (http://digit03.africamuseum.be), covering the Dacini (105 species), Ceratitidini (59 species) and Gastrozonini (11 species) tribes. The host range is known for 10 Ceratitidini and 42 Dacini species. The second data set included individuals of both sexes reared in La Réunion from the generalist species *Bactrocera dorsalis* (n=60), *B.* (n=14), *Ceratitis capitata* (n=60), *C. catoirii* (n=14), *C. quilicii* (n=14), the Solanaceae specialist *Neoceratitis cyanescens* (n=14) and the Cucurbitaceae specialists *Dacus ciliatus* (n=14), *D. demmerezi* (n=60) and *Zeugodacus cucurbitae* (n=14).

Results: After a non-linear body size normalisation, we found significant sexual dimorphism: females had larger funiculus surfaces than males (ANOVA, $p<10^{-8}$). In addition, we found that the ratio of funiculus to eye surface correlated with the host range (ANOVA, $p<10^{-15}$). It was lower for generalist species than for specialist species both in the Dacini and Ceratitidini, and was larger for the Cucurbitaceae specialists than for other specialists.

Conclusions: We found robust evidence that, regardless of phylogeny, generalist species have bigger eyes and Cucurbitaceae specialists have larger olfactory epithelium than other frugivorous Tephritidae species. This suggests, respectively, improved visual and olfactory acuity. To find the various hosts of generalist species with diverse volatile emissions, selection pressure might be stronger on the visual than on the olfactory modality. As for the Cucurbitaceae specialists, they could have evolved higher olfactory sensitivity to detect dull hosts with low levels of volatile emissions. Overall, our finding might help focus design efforts for future trap development. In addition, the sex-specific difference we observed supports prolonged efforts to discover new female lures.

***Keywords*:** morphology, host range, olfaction, vision, sensory organs, antenna.

Diet development of the Cape fly *Ceratitis quilicii* (Tephritidae)

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Background: *Ceratitis rosa* and *C. quilicii* (Cape fly) are two important fruit fly pests occurring throughout South and East Africa. The two species were still considered to be a single taxon (*C. rosa* or Natal fly) up until 2016, at which point sufficient data separated them into two taxa. For rearing of *C. quilicii*, various diets, supplements and protocols need to be adjusted to adapt rearing protocols for this species, which appears to have different requirements from *C. rosa.* Nutrition during developmental stages of fruit flies, especially larval stages, influence their development which will affect their size, shape and fitness. The following study evaluated the effect of different artificial larval diets with variable protein content on different physiological and morphological parameters of *C. quilicii*.
Methods: The effect of three different artificial larval diets, containing primarily dehydrated carrot powder and brewer's yeast (4, 8 and 12% protein) were evaluated in terms of the following parameters: body composition (water, protein, lipid and carbohydrate); pupal weight and body weight; tempo of emergence; starvation and desiccation resistance; and wing morphometrics.
Results: A canonical variate analysis showed clear differences of wing shape between the different diets but were not well separated. Significant shape changes caused by nutrition, warrant further investigation. For the physiological parameters, differences were also observed between the different treatments, with 8% diet showing higher desiccation and starvation resistance, higher emergence and lower adult weight.
Conclusion: Clear differences between the different treatments of the different parameters measured indicate that adjustments will most likely be needed to improve performance and fitness of reared *C. quilicii*. Further research should focus on measuring flight performance of these flies on the various diets.

Keywords: Cape fly, mass-rearing, IPM, physiology, morphology, adult fitness

Session 2: Taxonomy and Morphology, November 14, 2022, 14:30 - 16:30

Elusive species delineation of *Bactrocera dorsalis* and *B. occipitalis* in the Philippines

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Background: The sibling species of fruit flies, *Bactrocera dorsalis* (Hendel) and *B. occipitalis* (Bezzi) are considered economically important pests in the Philippines because they pose threats to horticultural production and trade. They are sympatric in the Island Garden City of Samal where the pilot study of areawide pest management funded by the Australian Centre for International Agricultural Research (ACIAR) is being conducted. *Bactrocera occipitalis* was identified as the dominant species in the year-round monitoring on the island. However, *B. dorsalis* is typically the dominant species in other parts of the Philippines. This study investigated the morphological and molecular characteristics of the local population and related them to similar studies of other local populations.

Methods: Fruit flies were reared from infested fruits and sorted based on the modified morphology rating system from White and Hancock (1997). The presence or absence of microtrichia on the thorax were also noted (Barr, et.al, 2021). Morphologically identified *sensu* White and Hancock *B. dorsalis* and *B. occipitalis* were subjected to molecular analysis using the internal transcribed spacer 1 (ITS1) region of nuclear ribosomal DNA and period genes. The congruence of the morphological characters and molecular identity of the flies was analyzed. Supporting studies on behavior and hybridization were conducted under laboratory conditions.

Results: The morphologically identified *B. dorsalis* and *B. occipitalis* matched the rating system of White and Hancock (1997). Molecular analysis of these flies showed that the consensus ITS1 sequences were 99.77% and 99.37% identical with the *B. dorsalis* sequences deposited in the National Center for Biotechnology Information, respectively. Further assessment using the microtrichia characters suggested by Barr, et al. (2021) showed congruence to the morphologically identified *B. occipitalis* but only 20% congruent to morphologically identified *B. dorsalis.* The inconsistencies in the morphological and molecular identification of the two sibling species were also observed in other local populations in the Philippines. Attempts to delineate the two sibling species using the combination of morphological characters with isozyme analysis, microsatellite marker analysis and DNA barcoding were unsuccessful. Species-specific host recognition was also not observed in laboratory studies. Interspecific hybridization of *B. dorsalis* and *B. occipitalis* was successful for up to 12 generations. The molecular identity and morphological attributes of the hybrids did not always match.

Conclusion: *Bactrocera dorsalis* and *B. occipitalis* could not be delineated morphologically and molecularly because of the gene flow between the two species. Conspecifics of *B. occipitalis* from Malaysia, Borneo, and Palawan should be examined to verify these results.

Keywords: ITS1, hybridization, species-specific host recognition, sibling species

Session 2: Taxonomy and Morphology, November 14, 2022, 14:30 - 16:30

Investigating drivers of speciation in the fruit fly genus, *Bactrocera* (Diptera: Tephritidae)

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Background: Traditional concepts of speciation suggest that lineages diverge through either substantial periods of geographic isolation or by adaptive radiation where a population takes advantage of a novel niche habitat. Studies on herbivorous insects suggest transitions onto novel host plants and resource partitioning have contributed to high levels of insect diversity, with populations specialising to overcome plant defences and to avoid competition with related taxa. *Bactrocera* is a highly diverse genus of frugivorous insects with over 450 species described predominantly from the tropical and subtropical rainforests of South-east Asia, Australia, Papua New Guinea (PNG) and the South Pacific. Contrary to theoretical predictions of how this diversity may have arisen, many *Bactrocera* species have high levels of polyphagy with both host-range and geographic-range overlap between species. Here we report on the use of diversification rate modelling techniques on an existing molecular phylogeny to provide insight into the potential drivers of diversification within *Bactrocera*.

Methods: The Bayesian Analysis of Macroevolutionary Mixtures (BAMM) model was used to estimate the speciation and extinction rates throughout an existing dated phylogeny which included 84 species from the three Dacini genera, *Bactrocera*, *Dacus* and *Zeugodacus* from Australia, PNG, and the South Pacific. Average speciation and extinction rates were compared between genera and clades within the phylogeny where significant shifts in speciation rates occurred were identified.

Results: The average speciation and extinction rates for *Bactrocera* at 0.14 new species per million years and 0.11 extinct species per million years were considerably higher than *Dacus* (0.07 and 0.03) and *Zeugodacus* (0.07 and 0.03). *Dacus* and *Zeugodacus* speciation rates decreased steadily over time, however a significant acceleration in speciation rates was observed in *Bactrocera* approximately 25 million years ago. The timing of this shift coincides with the Australian continental plate colliding with the Asian continental plate. Our findings support existing biogeographic hypotheses that flies dispersed from Asia into Australia and the Pacific. A potentially significant observation of these results is that 82% of species within the clade that experienced the accelerated speciation rate are host-use generalists, compared to 62% of species outside of the post-shift clade.

Conclusion: Estimating historical speciation and extinction rates within *Bactrocera* has provided insight into the potential drivers of diversification. The expansion into southern PNG, Australia, and the South Pacific, as well as maintaining a wide host breadth, appear to have played a significant role in the diversification of the genus.

Keywords: *Bactrocera*, speciation**,** diversification

Session 2: Taxonomy and Morphology, November 14, 2022, 14:30 - 16:30

New molecular phylogeny and diagnostic tools for *Anastrepha*

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Background: The taxonomy and identification of species of *Anastrepha* has largely relied upon characters of the adult female; males and immature stages of many species are difficult to impossible to distinguish morphologically. Phylogenetic relationships among the species are also incompletely understood.
Methods: To develop more rapid and reliable diagnostic tools for all life stages and a more robust phylogeny, our project, funded by the USDA Farm Bill and Plant Protection Act 7721, sought to improve upon existing morphological and molecular identification methods for both adults and larvae. Our efforts also included extensive collaboration with colleagues and agencies in countries throughout the range of *Anastrepha* to obtain a comprehensive set of samples for analysis.
Results: Sampling efforts included extensive surveys in Brazil, Ecuador, Peru, and Suriname, and shorter collecting trips in Bolivia, Colombia, French Guiana, and Panama funded by USDA, and contributions from multiple collaborating agencies (AGROCALIDAD, Ecuador; ICA, Colombia; MIDA, Panama; SENASA, Peru; SENASAG, Bolivia; SFE, Costa Rica) resulting in an extensive set of fresh samples in 95% ethanol suitable for molecular study. More than 50 previously unrecognized species have been formally described, and numerous additional species discovered. These new species and additional data and illustrations are being added to the interactive adult key. New larval morphological characters have been discovered, descriptions of larvae of 11 species have been completed, and an interactive key is in preparation. An extensive, verified COI library for the genus (2650 sequences, 273 species) has been developed and analyzed; COI barcodes are sufficient for diagnosis 206 of the 273 species included. Rapid diagnostic methods targeting ITS1 and other loci are being examined for their utility to identify the major pest species. The phylogeny developed using Anchored Hybrid Enrichment methods, based on hundreds of nuclear genes, has resolved many of the uncertain relationships within *Anastrepha* and corroborates the inclusion of *Toxotrypana*. It has helped to resolve some questions within the *fraterculus* complex. Analysis of the data is continuing to determine which additional DNA regions are most useful for diagnosis.
Conclusion: The diagnostic tools resulting from this project significantly enhance capabilities for the identification of all life stages of most species of *Anastrepha*. Further investigation of some taxa missing from the data set and further analysis of some DNA regions discovered via the AHE analysis that appear useful for diagnosis are needed.
Key Words: *Anastrepha*, phylogeny, taxonomy, diagnostics, identification

Session 2: Taxonomy and Morphology, November 14, 2022, 14:30 - 16:30

Revision of the *Bactrocera tryoni* species complex

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Background:
The *Bactrocera tryoni* complex is a sibling species complex that currently represents four morphologically and genetically similar species: the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), the lesser Queensland fruit fly *B. neohumeralis* (Hardy), the Northern Territory fruit fly *B. aquilonis* (May), and *B. melas* (Perkins & May). *Bactrocera tryoni* is the most economically important member of this complex, occurring across the east coast of Australia and New Caledonia, and recorded from 200 host plants in 49 families. Despite the long taxonomic history of this group, the specific status of some members of this complex have historically been called into doubt, while phylogenetically the complex has never been reviewed.

Methods:
We analysed existing and new morphological and molecular evidence for members of the complex collected from 16 locations across Australia, Papua New Guinea, and the South Pacific. A combination of morphological hypothesis testing, Sanger sequencing data, and DArTSeq genome-wide SNP data were used to evaluate the species status of members of the group. Alongside traditional taxonomic characters, seven diagnostic loci and >25,000 SNPs aided in species/complex delimitation.

Results:
Genetic data supports the addition of *B. mutabilis* (May), *B. ustulata* Drew, *B. curvipennis* (Froggatt) and *B. erubescentis* (Drew & Hancock) in the complex; whilst a combination of morphological and molecular data supports the synonymization of some members of the group, notably *B. melas*. We also synonymized *B. humilis* Drew & Hancock with *B. tryoni*; a species not previously placed in the complex, but described from a single individual. Based on these findings, we re-define the taxonomic bounds of the complex to now include: both monophagous and polyphagous species; species attracted to both cue-lure and isoeugenol; and species native to a wider geographic range. Additionally, we discuss the paraphyly of *B. neohumeralis* in the context of geographically disparate host use data across its native range of Australia and Papua New Guinea.

Conclusion:
Greater resolution of the *B. tryoni* species group using molecular data provides clarity on the limits of the group and species within it, with potential implications for management, trade, and biosecurity.

Keywords: *Bactrocera tryoni* complex, taxonomy

Session 3: Genetics and Biotechnology, November 15, 2022, 08:30 - 10:35

Global population genomics of Oriental fruit flies using RAD-seq

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Background: Determining the geographic source of Oriental fruit flies detected in surveillance or at inspection sites where it is not established requires a firm understanding of the genetic diversity and population structure throughout its established range. Prior studies using mitochondrial genes and microsatellite data detected structure when comparing native versus introduced populations, but structure within the native range across mainland Asia and Southeast Asia has not been adequately resolved. It is possible that repeated movement of fly-infested fruits and flight has contributed to loss of structure. To test the population structure of the Oriental fruit fly using genomic data, a restriction enzyme associated DNA sequencing (RAD-seq) technique is applied to the problem.

Methods: A total of 1,740 flies were included in the study. The majority were collected from traps set in native forest and agricultural areas throughout Southeast Asia between 2010–2019. DNA was extracted for flies using Qiagen DNeasy Blood & Tissue kit or a NucleoMag Tissue Kit. GBS libraries were prepared using the restriction enzyme *EcoT22I*, and sequenced on both an Illumina HiSeq 2000 and Novaseq 6000 as single-end (SE) reads. Stacks v2.41 were used to detect homologous loci throughout the genome, genotype SNPs, and filter SNPs. Population genetic structure was examined using STRUCTURE, discriminant analysis of principal components (DAPC) using ADEGENET 2.0., and AMOVA analysis in GENEPOP. Genetic connectivity among genetic populations was estimated using MOMENTS.

Results: The Illumina RAD sequencing generated over 100 million reads. A total of 244,889 loci aligned to the *B. dorsalis* genome. Further filtering reduced the number of informative SNPs to 1944 for 1740 individuals. At K=7, Structure detected seven clusters: (1) Africa, (2) Hawaii, (3) Sri Lanka, (4) Insular region (Malaysia, Indonesia, and Philippines), (5) Mainland Asia (Bangladesh, Cambodia, Vietnam, Thailand, and part of China), (6) Northern China, and (7) Southern China. The invasive populations in Hawaii and Africa had the greatest level of differentiation in analyses. The Asian populations were less differentiated. Significant levels of gene flow was detected among native populations.

Conclusions: The RAD-seq technology was successful at detecting structure in the species that was not measured using less data-rich methods. A new hypothesis of seven Oriental fruit fly populations is presented that can be used in development of source estimation studies of the pest. The technique described here could be applied to newer collections from areas that have not been sampled such as India to test and refine the hypothesis.

Keyword: RAD, SNP, source estimation, population genetics

Session 3: Genetics and Biotechnology, November 15, 2022, 08:30 - 10:35

Identifying translocation breakpoints in tephritid genomes

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Background: Genetic Sexing Strains (GSS) used in non-transgenic Sterile Insect Technique (SIT) colonies make pre-adult emergence sex-sorting and male-only releases possible in the tephritid species *Anastrepha ludens*, *Bactrocera dorsalis*, *Ceratitis capitata*, and *Zeugodacus cucurbitae*. Sex-linkage of selectable phenotypes in tephritid GSS were created artificially by chemically inducing reciprocal translocations between the selectable autosomal gene and a sex-determining locus on the Y-chromosome. These translocation breakpoints have been investigated using cytogenetic methods, but their precise genomic positions and sequences are largely unknown.

Methods: To further investigate stable sex-linked reciprocal translocations so that they can be recreated in tephritid species of emerging economic importance, we assembled the genomes of wildtype tephritids to chromosome-scale and generated chromosome-conformation (HiC) sequences for both wildtype and from their corresponding GSS. By comparing the wildtype chromosome-confirmation to the GSS, we were able to determine the genomic coordinates of sex-linked translocations in tephritid GSS.

Results: Analyzing the data for *Z. cucurbitae* revealed that the resulting data was largely in parity with existing karyotype and previous linkage-mapping data, though the high-throughput HiC sequences were able to resolve the breakpoints down to a higher resolution than in previous studies. In addition, the HiC data revealed a private inversion that was private to the GSS and not previously observed.

Conclusion: By comparing the wildtype chromosome-confirmation to the GSS, we were able to determine the genomic coordinates of sex-linked translocations in tephritid GSS. This will facilitate the development of new GSS with translocation-based sex linkage. Sequences at the identified translocation break points can be used to create CRISPR-based targeted double-stranded breaks in contrast to the traditional method of chemical mutagenesis which results in random double-stranded breaks in the genome. These targeted CRISPR-based methods, will result in GSS with fewer off-target effects that can be evaluated for production more swiftly.

Keywords: Sterile Insect Technique, Genetic Sexing Strains, Structural polymorphisms, Chromosomal translocations, Artificial sex linkage

Session 3: Genetics and Biotechnology, November 15, 2022, 08:30 - 10:35

Genetic diversity of *Bactrocera dorsalis* captured in California over a 14-year period

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Background:
In 2014, a publication by our group reported on the genetic diversity of *Bactrocera dorsalis* trapped in California using the mitochondrial *cytochrome c oxidase* subunit I (COI) gene. That study demonstrated that about half of the flies captured between the years 2008 to 2012 had COI types inconsistent with a Hawaiian source model. Since that study, our team has continued to sequence COI from flies trapped in California to evaluate the Hawaiian source hypothesis of each fly and document diversity of fly captures. In addition, a study using a SNP typing protocol was conducted to test if the Hawaii exclusion test is supported by the fly nuclear genome. This presentation provides an update on the records for a 14-year period, spanning from 2008 to 2021.

Methods:
DNA was extracted from the legs of 604 fruit flies that were morphologically identified as the *B. dorsalis* group and subsequently genotyped for *B. dorsalis* identification using the internal transcribed spacer 1 DNA. An approximately 800bp segment of the COI gene covering the 3′ end of the gene was amplified using PCR and sequenced using ABI Sanger-based technology. The edited data were trimmed to a length of 765bp, and then compared to determine diversity, similarity to Hawaiian population, and unique sequence types. In addition, a set of 48 SNPs designed from a RAD-seq dataset from pooled *B. dorsalis* samples, was used to independently test a Hawaiian source model of flies trapped in years 2008 to 2018. The 48 SNP panel was performed using a Fluidigm SNP typing assay on a microfluidic chip.

Results:
Of the 604 flies, 34 failed to generate COI data, 187 were consistent with Hawaiian population (7 sequence types), and 383 were inconsistent with a Hawaiian population (109 sequence types). New COI sequence types were observed every year. The SNP typing data showed a high level of agreement with the COI-based Hawaiian sources test.

Conclusion:
The genetic diversity of *B. dorsalis* captures in California over a 14-year period is relatively high and the COI-based exclusion test for a Hawaiian source is supported by additional nuclear genome methods. The study results provide additional evidence for multiple invasions of the pest into California.

Keywords: Diagnosis, COI, invasion, Oriental fruit fly

Mitochondrial haplotype diversity revealed multiple and fluctuating entry source of *Bactrocera dorsalis* complex into southwest Japan

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Background: Even after the eradication using MAT in 1986, the oriental fruit fly, *Bactrocera dorsalis* species complex (BDC), frequently entered into Japan, probably by long distance flight, and the number of the cases has been on the increase, especially in recent years. In order to capture the characteristics of the invasive species and population which may differ in from one to another and may provide useful information to prevent the establishment of BDC in the future, we conducted a morphological and genetic grouping of BDC that collected Okinawan islands to explore the relationship with the invasion pattern.
Methods: Dried or ethanol-fixed specimens (a total of 660 individuals) of BDC collected from Okinawan islands between 1978 to 2020, were used. ND4 (592 bp) regions of the mitochondrial DNA was amplified with PCR for direct sequencing analysis. Using the ND4 sequence registered in Genbank, it is possible to discriminate species (*B. dorsalis* or *B. occipitalis*) inhabiting the introduction sources (Taiwan, China, and the Philippines), which have been inferred from wind-borne immigration analysis. Additionally, for *B. dorsalis* specimens collected from the Philippines can be distinguished from those from other regions. Therefore, we attempted to identify the species and locality by creating a phylogenetic tree with the sequences registered in Genbank and those obtained in this study.
Results: In total, 580 specimens were successfully sequenced, obtaining 69 haplotypes. These included 561 individuals and 59 haplotypes of *B. dorsalis* (447 individuals and 25 haplotypes of the Philippine type and 114 individuals and 34 haplotypes of the non-Philippine type), and 19 individuals and 10 haplotypes of *B. occipitalis*. The case of entry and the proportion of the three groups fluctuated greatly from year to year, but years with high numbers of entry had high numbers of specimens from the Philippines, and year-to-year variation in non-Philippine types was small. The proportions of the three groups differed among the Okinawan islands and were roughly divided into three regional groups.
Conclusion: The present study suggests that (i) there may be more than one source of entry into southwestern Japan, (ii) at least for the period 2012-2020 when sample sequencing success was high, the Philippines is the main source of entry, and (iii) the proportion of entry from each source varies among the islands in Okinawa.

Keywords: the oriental fruit fly, Ryukyu islands, source of introduction

New generation of genetic sexing strains facilitate colony refreshment for SIT applications: proof-of-principle in medfly

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Background: Laboratory domestication triggers significant evolutionary changes in insect populations, including genetic sexing strains (GSS), which may affect their sexual behaviour. Long-established laboratory populations may have gone through several selection processes, for example genetic drift, bottlenecks, and inbreeding phenomena, which may have in turn modified their genetic structure and resulted in the reduction of their genetic diversity. Therefore, refreshment of the colonies of GSS, such as the *Ceratitis capitata* (medfly) VIENNA 8 strain, with wild material is frequently needed. However, this is a time-consuming and challenging process because wild males do not carry the translocation and wild females are not easily adapted to the artificial oviposition behaviour. To overcome these challenges, we present a new generation of GSS which facilitates the refreshment with wild genetic material, increasing genetic variability and potentially improving sexual performance.
Methods: Males from the mutant strain were irradiated to produce the translocation. Irradiated males were mass-crossed with wild-type females. Single pairs were then set up between F1 females and wild-type males. The established families were screened to select those that produced males with the wild-type phenotype and females with the mutant phenotype in subsequent generations. An additional selection step was applied to identify families in which the females could be eliminated thus establishing a novel GSS for further evaluation.
Results: Two lines were isolated and characterized in comparison to the standard T(Y;5) VIENNA 8 GSS. The new GSS has comparable or better quality and production profile than VIENNA 8 GSS under small scale rearing conditions. In field cage tests, males originating from the new GSS and VIENNA 8 GSS competed equally with wild males for mating with wild females. Five consecutive rounds of introgression were performed between females from both novel GSS with wild males from Spain and a follow-up analysis indicated a high degree of genetic refreshment.
Conclusions*:* Novel medfly GSS were constructed, which have similar characteristics with the T(Y;A)-based VIENNA 8 GSS. Their unique advantage is that they enable the smooth and controlled introduction of fresh genetic material into mass-reared colonies, potentially improving the quality of mass-produced males and the efficacy of medfly SIT action programs.

Keywords:
Medfly, genetic sexing strain, colony refreshment, genetic variability, translocation

Session 3: Genetics and Biotechnology, November 15, 2022, 08:30 - 10:35

Rapid Screening of Tephritid Surveillance Traps using DNA Metabarcoding

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Background: Routine surveillance for tephritid fruit flies is conducted globally to detect new introductions of exotic species, range shifts of established species, and monitor population levels for pest management. Depending on the target habitat and attractant lure used, each trap has the potential to collect hundreds of individuals from a range of species, with taxonomic identification of the resulting mixed specimens presenting a major bottleneck for timely management response. This research developed and evaluated a non-destructive metabarcoding approach, which simultaneously identifies bulk insects using DNA barcoding and retains intact specimens, to rapidly screen unsorted surveillance traps for multiple pest tephritids.

Methods: The optimal combination of diagnostic loci for discriminating lure responding dacine species was evaluated in-silico, then primers designed appropriate for contemporary HTS platforms. A tandem metabarcoding assay using the COI and EIF3L loci was then developed, and its sensitivity established using mock communities of increasing size. The qualitative and quantitative performance of the metabarcoding assay was then evaluated on real trap samples from routine surveillance operations across Australia that had previously been morphologically identified.

Results: The non-destructive metabarcoding assay was able to reliably screen approximately 500 specimens per sample, each containing up to 14 species, with up to 50 samples analysable in a single sequencing run. By circumventing all morphological sorting, the metabarcoding assay developed here may allow a substantial increase in the number of traps that can be processed simultaneously by a diagnostics lab. Furthermore, the assay showed semi-quantitative performance across the evaluated species, opening the scope for use in pest management applications where relative abundance of species needs to be compared over time or between treatments. When applied to real trap samples, however, some key pest species complexes could not be differentiated, highlighting the ongoing issue that close genetic relatedness and uncertain taxonomy presents for diagnostics of these economically important pests. In cases where target pests could not be unambiguously identified, the metabarcoding assay may need to be supplemented with targeted approaches such as qPCR to ensure high-priority exotic species are not being confused with common native species.

Conclusion: Despite the inability of the assay to discriminate some important pest species complexes, adoption of metabarcoding for high-throughput taxonomic screening of tephritid surveillance traps shows promise for reducing the technical and financial burden of surveillance, with the non-destructive DNA extraction approach allowing detections to be confirmed using traditional diagnostic approaches (i.e., morphologically) and specimens retained to support proof of freedom and market access negotiations.

Keywords: Dacinae, Molecular Diagnostics, Surveillance

Comparative olfactory responses of two major cucurbit pests, *Zeugodacus cucurbitae* and *Zeugodacus tau* (Diptera: Tephritidae) to three phenylbutanoids male lures

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Background:
Zeugodacus cucurbitae and *Z. tau* (Tephritidae: Dacini) are two major fruit fly pests of cucurbitaceous plants. Both species have similar geographical distribution and overlapped hosts range. Males of *Z. cucurbitae* and *Z. tau* are attracted to cue lure (CL), a common male lure used for population detection, monitoring and control programmes. Previous studies on other fruit fly species shown that fruit fly-lure response is species-specific and has great implication on the success of lure-based fruit fly control. Both species are also reported to attract to raspberry ketone (RK) and zingerone (ZN), both of synthetic and natural sources.

Methods:
We compared the species sensitivity of both *Z. cucurbitae* and *Z. tau* to CL, RK and ZN via Probit bioassays. Phenylbutanoid chemicals were serially diluted in absolute ethanol to yield 10 to 750 µg/10 µl. Sexually mature virgin males of both species were assayed separately in screen cages to different doses of chemical lures, starting with the lowest concentrations. Positive response was when flies responded by oriented flight and direct feeding on chemical source dispensed on a piece of filter paper within 10 min. Absolute ethanol was used as controls. Bioassays were conducted from 09:00 to 11:00 hr, which is the peak period of flies' response to chemical lures. Experimental procedures were replicated five times for different species and chemical lures on different days. Data were subjected to Probit analysis using PoloPlus software.

Results:
The ED_{50} of *Z. cucurbitae* to CL and RK was 75.0 µg and 152.6 µg while *Z. tau*, at 85.4 µg and 261.5 µg, respectively. Results showed that *Z. cucurbitae* is at least 1.14 and 1.71 more sensitive to CL and RK than *Z. tau*. The time taken for *Z. cucurbitae* to respond and to arrive at the chemical source tested was also shorter than *Z. tau*. The ED_{50} of both species to ZN could not be determined as the heterogeneity factor of the Probit assays was high (2.5-2.8).

Conclusions:
Probit assays revealed that although *Z. cucurbitae* and *Z. tau* respond better to CL than to RK, *Z. cucurbitae* is more responsive than *Z. tau* based on the ED_{50} values obtained. This results commensurate with the current practice of using CL as the most effective male lures for *Z. cucurbitae* and *Z. tau* in fruit fly population detection, monitoring and suppression programmes.

Key Words: *Zeugodacus cucurbitae*, *Zeugodacus tau*, cue lure, raspberry ketone, zingerone

Session 4: Chemical Ecology and Attractants, November 15, 2022, 11:00 - 13:00

Determining the presence and abundance of oriental fruit flies is more complex than counting numbers in traps

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Background: Key to the maintenance of fruit fly free areas or areas of low fruit fly prevalence is effective surveillance. The presence and risk of infestation by fruit flies, including oriental fruit flies, *Bactrocera dorsalis*, is mostly determined using trapping. Threshold levels have been established for this pest but trapping levels depend not only on insect abundance but also environmental conditions and the responsiveness of the flies to the attractant, methyl eugenol (ME). It is already known that only older, sexually mature males respond to ME, reducing the capacity to detect females and young males. We have been evaluating the environmental variables affecting the responses of *B. dorsalis* males to ME, with a focus on the effects of temperature on lure formulation loss, activity, and trap captures.

Methods: We placed commercially-available ME polymeric plug dispensers (Chempac ME Lure, Chempac, Suider Paarl, South Africa) in yellow bucket traps along the northern and southern edges, and in the centre of a lemon orchard at the University of Pretoria, South Africa, and measured temperature and relative humidity in these locations. Weekly weight loss by the ME dispensers over a one month period was measured. This was repeated over six months and ME dispenser weight loss was related to mean weekly environmental conditions. In the laboratory, we went on to record undisturbed behaviour (inactivity, grooming, walking, flight) and tethered flight of *B. dorsalis* between 12 and 36˚C. We then ran field cage studies to determine the proportion of a known number of flies caught in ME-baited traps under varying field temperatures.

Results: The ME dispensers used in traps lost more weight at temperatures above 20˚C. This temperature also represents a threshold for optimal walking and flight performance of *B. dorsalis*. In field cages, sexually mature male *B. dorsalis* were less likely to be caught in ME-baited traps when temperatures were cool, with no trap captures expected below 21.2±1.8˚C.

Conclusion: Our research shows clear effects of weather on fly attraction to ME. The outcome of this is that *B. dorsalis* males are less likely to move towards and be caught in ME-baited traps when temperatures are cool but well above those that cause mortality. This necessitates that thresholds of ME-based trap captures that trigger management actions, including quarantine, need to be adjusted according to prevailing environmental conditions.

Keywords: *Bactrocera dorsalis*, Flight, Surveillance, Temperature

Session 4: Chemical Ecology and Attractants, November 15, 2022, 11:00 - 13:00

Fruit flies (Diptera: Tephritidae) olfactory systems specialize in discriminating their various hosts

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Background: Tephritidae fruit flies are among the major pests of fruit and vegetable crops. The control of these pests is mainly based on chemical products. Alternative ecologically sustainable strategies integrate a set of measures, including methods based on insect olfaction. However, the olfactory strategies used by Tephritidae species are still unclear for defining effective control methods. Thus, this study aims to investigate the sensory mechanisms used by polyphagous Tephritidae species to recognize their host-fruits. For generalist species, it is generally thought that among the various compounds emitted by the wide range of hosts, insects rely primarily on those shared by most hosts. To challenge this hypothesis, we conducted studies based on evolutionary, chemical ecology and ecoethology approaches.

Methods: Firstly, we analysed the volatile emissions of 28 fruit species using gas chromatography couple with mass spectrometry (GC-MS, n = 7 per species). For each volatile compound, we estimated its sharedness among fruit species with an index of fruit □-diversity. We also established a phylogenetic reconstruction on these fruits by Bayesian inference and performed a 'phylogenetic principal component analysis' (pPCA) of their emissions.
Secondly, we assessed the olfactory sensitivity of 8 Tephritidae species, including the invasive oriental fly *Bactrocera dorsalis*, to 37 synthetic compounds with various degrees of sharedness among fruits by GC coupled with a triple electroantennogram detector (n = 8 per species).
Lastly, behavioural tests were carried out on *B. dorsalis* with a dual-choice *ex-situ* trap. We compared the attractivity of two synthetic compound blends at low and high concentrations, namely a blend of 5 fruit-specific compounds (low sharedness), a blend of 5 compounds with high sharedness among fruit species.

Results: With GC-MS analysis, we identified 511 compounds from intact fruits and 665 from sliced fruits. pPCA highlighted the compounds specific to phylogenetic groups of fruits, while a comparison with the α-diversity index revealed that sharedness is not correlated with fruit phylogeny. Then, we found for each fruit fly species a significant negative correlation between the olfactory sensitivity of flies and fruit α-diversity. Accordingly, a behavioural bioassay showed that at low concentration *B. dorsalis* prefers compounds specifically emitted by few fruits species than compounds that are shared among fruits.

Conclusions: This unexpected result supports a new hypothesis: Tephritidae species are not only specialized in detecting their hosts, but also in discriminating them. Optimization of host-discrimination would have resulted in low sensitivity threshold for specific compounds and wider dynamic range for shared compounds. This new concept implies many selective advantages, including adaptation to the local background, and should also drive the search for more effective attractants.

Keywords: *Bactrocera dorsalis*, Chemical ecology, Electroantennography, Olfactory system.

Session 4: Chemical Ecology and Attractants, November 15, 2022, 11:00 - 13:00

Identification of a male-produced aggregation sex pheromone for *Bactrocera minax* (Enderlein) and a host attractant for male *Bactrocera tsuneonis* (Miyake)

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Background: Chinese citrus fly, *Bactrocera minax*, is a major citrus pest in many citrus production areas in China, and the Japanese orange fly, *Bactrocera tsuneonis*, is an important pest of citrus in Japan and is also present in southwestern China (Diptera: Tephritidae). Both species are univoltine and overwinter as pupae in the soil. Two highly attractive kairomone lures used for many other *Bactrocera* species, cuelure or methyl eugenol, are not attractive to these fly species. Hydrolyzed protein, sugar, and vinegar mixtures are only marginally effective in attracting adult flies. With increased global passenger movement, there is a possibility that fresh, infested citrus fruits could be a pathway for these flies into the U.S. Currently there are insufficient data on effective lures and survey tools for early detection of Chinese citrus fly and Japanese orange fly. The present study addresses this lack of knowledge by investigating possible pheromone and host odour attractants.

Methods: Wild-collected *B. minax* and *B. tsuneonis* pupae were shipped from China to the Forest Pest Methods Laboratory quarantine. Virgin flies were sexed and kept separately, and fed nitrogen-containing diets mixed with sucrose. Volatiles were collected from males, females, and unripe sweet oranges (*Citrus sinensis*) at 2-3-day intervals. Collected volatiles were analysed and compared by GC-MS and GC-EAD. Compounds with antennal activity were quantified and identified by GC-MS, GC-FID, and verified by authentic standards. For *B. minax*, ethyl (*Z*9)-hexadecenoate (10 mg), ethyl hexadecanoate (0.83 mg), ethyl-tetradecanoate (0.5 mg) were formulated on dental wicks. For *B. tsuneonis*, seven GC-EAD active compounds were formulated into micro polyethene vials; (R)-+-limonene (37.5 mg), (+/-)-linalool (37.5 mg), 4-terpineol (18.75 mg), α-terpineol (37.5 mg), (*Z*)-citral (18.75 mg), (*E*)-citral (18.75 mg), geranyl acetate (6.25 mg). Replicated field tests in China consisted of lures placed on green sticky sphere traps.

Results: Male *B. minax* released a sex-specific blend of 3 ethyl esters that increased over time, peaking just before sexual maturity. Both male and female antennae responded to this ethyl ester blend in GC-EAD tests. Male and female *B. minax* were significantly attracted to the ethyl ester blend in the field. Male and female *B. tsuneonis* antennae responded to seven compounds collected from unripe sweet oranges. The synthetic sweet orange blend was highly attractive in the field, but only to male *B. tsuneonis*.

Conclusion: We identified a male-produced aggregation sex pheromone for *B. minax* consisting of ethyl (*Z*9)-hexadecenoate, ethyl hexadecanoate, ethyl-tetradecanoate and a host odour attractant for male *B. tsuneonis* flies.

Keywords: *Bactrocera minax*, *Bactrocera tsuneonis*, aggregation sex pheromone, host odour attractant.

The effect of various physiological parameters on the response of *Bactrocera zonata* males to methyl eugenol

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Background:
The attraction of several *Bactrocera* species males to Methyl eugenol (ME) is a critical element in the control strategies. The control of the peach fruit fly, *B. zonata*, is mainly based on the Male Annihilation Technique (MAT), which lures the males to ME baits depleting their population and leaving the females unmated. The Sterile Insect Technique (SIT) against *B. zonata* is a potential option to be considered, but due to the essence of MAT it conflicts with the release of sterile males. A possibility to combine both techniques require the reduction of the sterile males' response to ME. The aim of this study, thus, was first to study the response of males to ME an to find way to reduced their attraction.

Methods:
The flies were obtained from laboratory colony established from flies that emerged from infested fruits collected in backyards in the metropolitan area of Tel Aviv and kept under quarantine restrictions in the Plant Protection and Inspection Services in Bet Dagan, Israel. For studying the response to ME we developed three bioassays: two indoor assays for short distance response and one attraction assay, in semi-field conditions.

Results:
A direct contact to ME was found to be toxic to *B. zonata*. Exposure of eggs and pupae to volatiles of ME, had no effect on the adult response. Pre-exposure of adult males, significantly reduced their later attraction to ME for at least 4 weeks. Males that were developed in artificial diet, responded significantly more to ME than males that were developed in fruits. The effect of sub-lethal dose of 0.2% (w:w) ME, decreased pupation on one hand but also significant lower ME-response of the survived males. Addition of hydrolysed yeasts to the adult diet, significantly increased the male's response to ME.

Among five different populations of *B. zonata*, 28.3 ± 4.5% (mean ± SE) of the males were 'indifferent' to ME. Selection of an "ME-indifferent" strain, did not further depleted this response, probably since it is not genetically related.

Conclusion:
Our findings show that the male response to ME can be manipulated to gain males that are less responsive to ME. However, strain selection may not be an option. A possibility to harmonize SIT/MAT as a control strategy for the peach fruit fly may be by using the concepts of aromatherapy, based on exposing sterile adult males to aroma of ME before release.

Keywords: para-pheromone; MAT; SIT;

Updates on the development of female attractants for improved monitoring of oviposition-impending *Bactrocera dorsalis*

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Background: There is a clear need for a new and more potent attractant for surveillance of female oriental fruit fly (OFF). Worldwide, OFF is one of the most destructive invasive pests of fruit that can become a serious trade-barrier once established. Current OFF surveillance programs in the US rely on a large number of traps (10,000 traps/yr in CA) baited with methyl eugenol and liquid protein bait (torula yeast, TY). Although methyl eugenol is a highly potent attractant, it only attracts males and cannot monitor OFF females. TY-baited traps can attract both sexes and thus have been used for detection of female OFF. However, TY is not strong enough to meet program needs as a standard female attractant; furthermore, TY traps are difficult to maintain, attracts many non-target insects, and changes attractiveness with time.
Methods: We evaluated differences in egg load and oviposition by mated female OFF that preferred host fruit odor or protein food odor. We collected headspace volatiles from a preferred host fruit (guava), then used GC-EAD and GC-MS to identify host fruit volatiles that can be detected by female OFF antennae. We evaluated the attractiveness of EAD-active compounds using laboratory bioassays and field trapping experiments.
Results: The lack of suitable sensitivity of TY traps for OFF females appears to be related to its inability to attract females ready for oviposition. In bioassays using cohorts of 14~16-day old mated OFF females, some preferred traps baited with TY while some preferred traps baited with host fruit. Interestingly, the mated females that preferred host fruit odor had 2X more mature eggs in their ovaries and laid 2X more eggs than mated females that preferred TY odor, suggesting great potential for using host fruit odor-based lures to monitor "oviposition-ready" mated females and improve the efficacy of OFF detection. We identified a 16-component chemical lure that is as attractive to female OFF as a preferred host fruit and significantly more effective than TY in catching oviposition-impending females in the field.
Conclusion: Our results suggest that protein-odor based OFF attractant will be preferentially more effective for unmated and not oviposition-ready mated female, while host fruit odor based lure is more sensitive to oviposition-ready mated OFF females. Thus, using both types of lures targeting OFF females with different reproductive physiological status will increase detection sensitivity of OFF females. In this talk, brief updates on other chemical ecology projects currently being conducted to develop synthetic protein based lure and oviposition deterrents for OFF.

Keywords: female attraction, food choice, host choice, physiological status

A forecasting system for fruit fly biosecurity and pest management

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Within the FF-IPM project we have created a multi-scale pest alert system to provide useful information for targeting biosecurity surveillance, incursion management and post-border pest management. Initially, the system is populated with models for three fruit flies: *Bactrocera dorsalis, B. zonata* and *Ceratitis capitata.* The models are driven by weather data drawn from the ECMWF and includes a combination of historical and forecast data. The forecasting system operates on a cloud-computing platform. CLIMEX models are used to estimate climate suitability in source countries. These data are combined with historical interception data and fly presence information overseas to generate country lists to prioritise fruit and vegetable consignments for inspection in conjunction with dynamic climate suitability maps. Dynamic weekly climate suitability webmaps are overlain with trap catch data to inform pest management. These traps are focused on the early detection of fruit fly activity. DYMEX population dynamics models are used to highlight fruit fly phenology and abundance, allowing pest managers to target treatments in space and time.

Keywords: pest alert system, pest forecasting, decision support, quarantine, climatic suitability, phenology, trapping, web-mapping

Development of microsatellite markers for the Japanese orange fly, *Bactrocera tsuneonis* (Diptera: Tephritidae)

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Background: The Japanese orange fly, *Bactrocera tsuneonis*, is one of important pest of citrus fruits. Although the distribution of this species is restricted to Japan, southern China, and Taiwan, there is a risk of its expansion to other areas. Although the Japanese government promotes the export of citrus fruits from by, several countries require quarantine for *B. tsuneonis* and permit imports only from unaffected areas. Therefore, to monitor and control this species, it is important to determine its dispersal ability. Since information about the genetic structure and geographical variability of this pest species could contribute to the prediction of its mobility, the development of molecular markers is crucial for the pest management of *B. tsuneonis*. To this end, we developed microsatellite markers at 17 loci for *B. tsuneonis.*

Methods: *B. tsuneonis* were obtained from Satsuma mandarin orange fruits randomly collected from several orchards in two cities of Oita, Japan. Genomic DNA was extracted individually from each fruit fly. All data obtained using the genetic analyzer were genotyped using GeneMarker® software. Genetic polymorphisms within populations were estimated in terms of the number of alleles, observed heterozygosity, expected heterozygosity, null allele frequencies, and inbreeding coefficient of the microsatellite markers in the adult populations of *B. tsuneonis* using GenePop and FSTAT.

Results: A total of 80 adult females and males randomly collected from Oita and Yamaguchi, in the western part of Japan, were used for polymorphism analysis using the microsatellite markers. The average number of alleles was 2.65 for the 17 loci, in which two loci did not show polymorphism. The null allele frequencies varied among populations. These results suggested a high degree of inbreeding for this species. Significant genetic differentiation between the Oita and Yamaguchi populations. A bottleneck effect was detected in Yamaguchi but not in Oita, and this is congruent with the history of an expanding distribution in this species.

Conclusion: We developed 17 polymorphic microsatellite markers for *B. tsuneonis* but did not detect a difference in population structure within a limited area. Analysis of population structure over a wider area must be performed to elucidate the population structure in the future.

Keywords: Japanese orange fly, microsatellite markers, population structure

General Guidelines to Facilitate the Opening of International Markets for Fruits and Vegetables that are Fruit Fly Hosts based on International Standards for Phytosanitary Measures

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This document was prepared for IAEA to explain aspects of international standards that facilitate the safe trade of fresh fruits and vegetables known to be fruit fly hosts. It explains key concepts and relationships within the regulatory framework that is defined by the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) and the International Plant Protection Convention (IPPC) with its associated International Standards for Phytosanitary Measures (ISPMs). Relevant provisions of the agreements, standards, and related disputes are explained in the context of risk management applications for fruit flies. The guide aims for a better understanding of the factors that should be considered when establishing trade, the important role of research, and how to maximize the potential for international standards to facilitate safe trade. It describes an evolutionary design for risk management to guide future efforts by the fruit fly community.

A central theme of the guide is the importance of being thoughtful about program objectives and the designs that achieve those objectives in a sound and defendable way in concert with international standards, recognizing that standards are incomplete and constantly evolving. This points to the importance of not only understanding and following standards, but partnering with the regulatory community to drive the evolution of standards and move pest risk management for fruit flies into a new era. No group is in a better position to advance this agenda than the fruit fly community because of its long history, broad foundation, and close relationship to the regulatory community. The guide emphasizes moving toward a holistic approach through close coordination with all relevant authorities, institutions, and organizations, including the research sector, regulatory authorities, producer and export associations, investors, and international organizations.

Keywords: Safe trade, Fruit fly risk management, International Standards for Phytosanitary Measures

Developing genus-specific generic cold treatments

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Background: Research on the efficacy of cold treatment disinfestation for fruit fly (Diptera: Tephritidae) has been ongoing for more than 100 years, but currently the only cold treatments approved as International Standards for Phytosanitary Measures (ISPM's) are restricted to two genera: *Ceratitis* and *Bactrocera*. To develop effective treatment schedules researchers have typically undertaken dose-response trials against all immature life stages and then undertaken large-scale trials against the most tolerant stage. But despite hundreds of treatment schedules being approved worldwide there are still no generic cold treatments approved and the debate on the most-cold tolerant life stage has not been conclusively resolved. While approved generic treatments tend to be overly conservative, in event of an exotic incursion they can allow trade to occur while shorter-time species/commodity specific treatments are developed.

Methods: A literature review combining historical data sets with recent large-scale trials was undertaken. Rather than model the thermotolerance of species the data was grouped by commonly approved treatment schedules (e.g., treatment of *C. capitata* in a range of commodities). The review also included findings from previous International Plant Protection Convention (IPPC) technical panel reviews and a meta-analysis of Australian research on the most tolerant stage of *C. capitata* and *B. tryoni*.

Results: There are inconsistencies in the published literature regarding the cold tolerance of tephritids. In some cases research on the same commodity, using the same temperature but in different laboratories, has resulted in different recommended treatment times. To overcome inconsistencies in treatment recommendations the development of genera-specific generic treatments must be based on treatment schedules for the most-cold tolerant species within a genera. While collated results clearly show that cold tolerance does vary between and within genera, and is dependent on the commodity being tested, the cold tolerance of *Ceratitis* and *Bactro*cera species is well documented and supports genus-specific generic cold treatments of 3°C for 23 days and 18 days, respectively. Both treatment schedules are highly conservative (2 to 6 days longer than published treatment schedules). The *Ceratitis* generic treatment also has potential to be expanded to cover *Anastrepha* and *Zeugodacus* spp, but more research on these genera is required.

Conclusion: A review of published and unpublished cold-disinfestation research allows for the recommendation that generic treatments of 3°C for 23 days for *Ceratitis* spp and 3°C for 18 days for *Bactrocera* spp be adopted. It is hoped that these recommendations, if approved as international standards, will provide more opportunities for cold to be used as a disinfestation treatment for trade.

Keywords: fruit fly, cold treatment, generic treatment, most tolerant stage

Host Reproduction Number for fruit fly management and trade

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Background: Fruit flies are the most significant insect pest in the fruit and vegetable industries. Most countries have endemic fruit flies to manage and to optimise fruit production. Importers need good quality and uninfested fruit, and exporters of traded fruit must create confidence that these commodities are not infested with fruit flies. Additionally, countries must be alert to incursions of exotic fruit flies and early warning surveillance is used by many countries.

These activities (surveillance, management, and trade) must continue in a world where the number of acceptable pesticides is declining, and where our climate is changing. So, the operating environment for fruit production and trade is changing, and historical management strategies are being challenged

It has long been known that different hosts have different capacities to support different levels of fruit fly populations. Historically, these hosts were called “good” hosts or “poor” hosts although the quantification of these terms was not done until recently. The host Reproduction Number (HRN) can service many work areas.

Methods: There is much historical HRN data available. We can review past papers and collate the existing knowledge on fruit fly species. Additionally, scientists need to collect data to support the HRN concept and add to the world knowledge. This includes weighing sample fruit and rearing out adult flies.

Results: Recently, the HRN for many species in the Pacific and in Africa were published. However, there are many gaps in the information for many species.

HRN has three important works areas. HRN can be used to optimise local management by targeting high HRN feral and commercial hosts. HRN can also inform surveillance for endemic and exotic incursions by identifying their preferred HRN hosts.

HRN can be used to assess import risk for fly-host commodities. For disinfestation treatments, not all hosts need to be treated with the same technique. Similarly, new trade protocols can be developed, particularly for low HRN commodities using the *Systems Approach.* An improved understanding of HRN for imports and exports may lead to less demand for treatments that add to climate change.

Conclusion: HRN has several areas where surveillance scientists, fruit fly managers and market access regulators could benefit from increased HRN knowledge. Hence, there is merit in scientists collecting more information in the HRN format for more commodities. In the future, HRN knowledge could assist tephritid scientists to change how they do their business.

Keywords: trade, management, surveillance,

Phytosanitary systems approaches

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Background: Phytosanitary systems approaches (PSA) are where two or more independent, phytosanitary measures are used to reduce the risk of pest and pathogen movement through trade. Although commonly used to manage fruit flies of quarantine concern, end point treatments remain the preferred option even where they have negative impacts on quality. An important reason is the difficulty in quantifying the efficacy of measures used in a PSA, individually and in combination.

Methods: We reviewed publicly available PSA protocols for fruit flies to identify which measures are most commonly used. We also developed a Pest Risk Reduction Scenario Tool (PRReSTo) to quantify the risk of fruit infestation, and to compare the ability of different permutations of measures to reduce risks to acceptable levels. We then applied this tool on an Australian case study (Queensland fruit fly in cherries) using data collected across several growing seasons.

Results: Thirty-nine unique measures were identified that can reduce risk in one of four ways: minimise exposure to the pest, reduce host vulnerability, reduce infestation rates and reduce establishment risks. Most of these measures have been used on fruit flies, although evidence to support their efficacy was frequently lacking. Field studies in the main cherry producing areas in Australia quantified the efficacy of several measures: limited seasonal overlap, block-based surveillance with a corrective action, crop-inspection (with suspension if an infested fruit is found) and packed-product inspection (with suspension if an infested fruit is found). Analysis using PRReSTo of cherry field studies found that the combined effect of these measures reduced risk of fruit infestation to very low levels.

Conclusion: PSAs offer considerable potential as a risk-based method for managing trade-related biosecurity risks. They are compatible with modern production and supply chains, and open opportunities to integrate newer technologies. Prospects include automated traps for cost efficient monitoring to 'minimise exposure to pests' and optical scanning which may offer a more effective measure to 'reduce infestation rates' in the consignment. Our review, modelling and empirical studies all suggest that there is considerable potential for further application of PSAs to fruit flies. The use of PRReSTo to quantify the combined effects of measures addresses an important impediment in the application of PSAs. However, to realise this potential, international agreement is also needed on how to demonstrate the efficacy of individual measures used in PSAs. Our improved methods can also be used to complement other pathways (such as phytosanitary irradiation) – for example to guide practices to minimise infestation risks prior to end point treatments.

Keywords: Market access; Phytosanitary Systems Approaches; Risk Management

Session 6: Sterile Insect Technique, November 17, 2022, 08:30 - 10:30

Development of the sterile insect technique to control the spotted-wing drosophila, *Drosophila suzukii*

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Background:
In less than 15 years, *Drosophila suzukii*, the Spotted-Wing Drosophila, has invaded several continents cause extensive damages to red and stone fruits. The control of this insect is particularly challenging because it infests fruit shortly before harvest, in the context of the gradual prohibition of broad-spectrum insecticides.

Methods:
The sterile insect technique (SIT) hence appears as a promising solution. Here we will present the state of the art, describing the major inputs made by several labs worldwide, include ours.

Results:
Optimal irradiation doses of pupae were rapidly identified. The rearing of the insect in large numbers however revealed more challenging: egg collection techniques and rearing media are still under refinement. Open-field tests of male release and recapture confirmed that, for *D. suzukii*, the SIT would be most suitable in confined or semi-confined spaces, such as greenhouses.
We selected a high performance strain of *D. suzukii*. When given the choice, wild females preferred mating with the high-performance strain rather than control strains, even when the first had been irradiated and the second not.
These encouraging results enabled semi-field tests in conditions of increasing realism. These showed even a ratio of 5 sterile males to 1 fertile sufficed to reduce females reproduction by a 3 fold factor.
Eventually, protection efficacy was tested in experimental greenhouses in which several dozens of strawberries plants were enclosed in netting and their production harvested like in commercial farms. After 45 days, less than 5% of berries were infested in presence of sterile males, while more than half of the berries were destroyed in control conditions.

Conclusion:
Collectively, the results presented here form the basis for a successful SIT program to control *D. suzukii* in confined or semi-confined conditions. Field trials in commercial farms will start in 2023 and provide real-life data on the efficacy of this solution.

Keywords: SIT ; Drosophila suzukii ; review

Session 6: Sterile Insect Technique, November 17, 2022, 08:30 - 10:30

Host plant volatile compounds capable of stimulating *Anastrepha fraterculus* male sexual competitiveness

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Background: In some tephritid fruit flies, exposure to volatile compounds from host plants increases male sexual success. This phenomenon has been proposed as a means to boost sterile males' sexual competitiveness in the framework of the sterile insect technique (SIT). Previous studies revealed that males of *Anastrepha fraterculus* (Diptera: Tephritidae) exposed to volatiles from guava (*Psidium guajava*) fruit (GF) and guava essential oil (GEO) exhibit intensified courtship behavior and have greater copulatory success relative to unexposed males. Similar results were achieved in these flies through exposure to moradillo (*Schinus polygama*) essential oil or lemon (*Citrus limon*) essential oil. The goal of this study was to identify compounds involved in the exacerbate male calling behavior that follows exposure to guava volatiles.

Methods: We identified the volatile chemical profiles of GF and GEO through chromatography coupled to mass spectrometry (GC-MS), and compared them with published profiles of moradillo and lemon essential oil. We selected compounds present in all profiles as candidate compounds. Using the electroantennographic detection (EAD) technique, we verified that males are able to detect all the candidate compounds between 0.01µg/µl and 100 µg/µl for each compound. Furthermore, we tested if exposure to each of these compounds stimulates male calling behavior at three doses (1, 10, and 100 □g/□l). We also tested two mixtures of candidate compounds for their effect on male calling behavior.

Results: We selected five candidate compounds: (E)-β-ocimene, (Z)-β-ocimene, limonene, β-caryophyllene, and α-humulene. Mature males' antennae were able to detect all candidate compounds. We confirmed a stimulating effect on the courtship behavior of males for (E/Z)-β-ocimene and (R)-limonene, whereas β-caryophyllene and α-Humulene did not affect male courtship behavior. For both β-ocimene and (R)-limonene the stimulating effect was dose-dependent. The behavioral response to these semiochemicals was further increased when β-ocimene and (R)-limonene were combined together in an exposure mixture. However, the response was reduced when β-caryophyllene and α-humulene were added to the mixture, which suggests negative interactions between some of the candidate compounds.

Conclusion: Our results support the potential of limonene, β-ocimene, or a mixture of both compounds as a stimulating factor capable of enhancing the sexual performance of *A. fraterculus* males. These compounds have yet to be tested on sterile males in the SIT context. Searching for these compounds in the volatile profiles of other, non-tested sources may help find other resources that can be employed to improve the SIT efficiency.

Keywords: semiochemicals, β-ocimene, limonene, calling, SIT

Predicting and achieving suppression of Queensland fruit fly using the sterile insect technique

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Background:
Queensland fruit fly, Qfly, *Bactrocera tryoni* is a serious pest of horticulture in Australia. The sterile insect technique (SIT) is currently being used against Qfly in Australia through the SITplus initiative. Substantial research advances have been made to improve Qfly quality within the production system from Phase 1 of the SITplus programme. Building on and implementing research from Phase 1 of the SITPlus programme, to effectively operationalise the field application of SIT flies, in Phase 2 we have predicted seasonal phenology of Qfly, modelled suppression and trialled the suppression of Qfly by using sterile mixed sex Qfly in a pilot release programme.

Methods:
Flies have been released over two townships over three and a half seasons. We have compared wild fly catch between release and nearby control sites. We have predicted and measured population suppression with sterile flies through time. Population suppression works best when a high ratio of sterile to wild flies are present. Knowing how many flies are needed at the time of ordering them when they are going to be released approximately two weeks later is a challenge. Using a Bayesian approach, we have attempted to predict the fly population two-weeks into the future so as to support growers to be able to order enough flies to reduce population density when they are ready to be released.

Results:
Catch of male Qfly was significantly reduced in townships where sterile flies were released over the three and a half years. We observed that catch in a trap was highly correlated with catch in that same trap the previous 1 and 2 weeks. Coupling this with temperature-driven phenology models, were able to reasonably predict magnitude of wild population increase and decline in advance. However, early in the season, the peak was predicted to occur a number of weeks earlier than what was observed. Later in the season, this timing and magnitude was better aligned.

Conclusion:
The approach we have taken here will support moves to optimise the number of sterile flies needed to suppress population growth throughout different times of the season. Improvements to the predictions may be made by modelling year round populations, including activity of over-wintering adults to ensure the initialisation at the beginning of the season is better timed.

Keywords: *Bactrocera tryoni*, model, phenology, sterile insect technique

Review of the main packing and release methods for sterile fruit flies around the world

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The methods of packing fruit flies not only include the different equipment such as paper bags, boxes or towers. It also considers the design of the packing center that will be used for the handling of the flies. The packing method also defines how the sterile fly releases will be made.

Packing Centers. Considering these criteria, we have Fixed and Mobile Packing Centers. It is of the utmost importance to select the place to establish the packing center. If the release is going to be aerial the center should be located close to an airport or airplane runway, preferably within the release area to avoid damage to the flies from long ferry time of the plane to the release area or of the ground release vehicle. Once the location of the center is defined, it will be necessary to select the type of fixed or mobile packing installation, based on the urgency for starting the use of the SIT. A fixed packinghouse takes more time to build.

Packaging Methods. A myriad of packaging methods are in use including cardboard and plastic boxes, plastic buckets, paper bags, mesh cages and towers. The towers have an automated packaging system. This facilitates its use and provides greater precision in the number of pupae deposited in each level of the tower, as well as a cleaner and safer use for the operator, using recyclable materials, such as pupae containers, food trays, resting areas and pads to provide water, which result in cost savings. The type of diet for adult fruit flies has two variations: those that use only sugar and the ones using sugar plus protein.

Release Methods. Release of sterile flies is normally carried out by ground or by air. Ground release primarily uses paper bags, plastic buckets, occasionally PARC boxes. While towers, net cages and PARC boxes are the most used for aerial release. The main modalities in aerial release are the use of endless screw or vibration band for dosing the flies when they leave the aircraft. Both modalities use a cooling system that regulates the temperature and humidity inside the transport box to keep the flies chilled. The boxes that use conveyor belts also have a vibration system that allows the flies to leave the box without mechanical damage and allows a uniform dosage. Both have equipment that allows the dose of sterile flies to be adjusted according to the needs of the control programme.

Navigation systems. There are two ways of working precision release. With a satellite flagger such as AgNav or SatLoc to define the area and bandwidth and another through the Macx toolset consisting of a Macx web and the MacxNav flagger.

Simultaneous Application of Sterile Insect and Male Annihilation Techniques to Enhance Fruit Fly Management

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Background: The male annihilation technique (MAT), which attracts and kills male insects, is an environmentally friendly pest control method and compatible with integrated pest management approaches. The MAT has been used to suppress and eradicate populations of certain fruit fly pest species in the genera *Bactrocera* and *Zeugodacus* (Tribe: Dacini). The sterile insect technique (SIT) is a target-specific pest control method without adverse environmental impacts. The SIT involves mass-rearing, sterilizing (with ionizing radiation), and mass-releasing sterile male insects to the target area to outnumber and outcompete their wild counterparts, and mate with wild female insects, resulting in non-viable offspring. In certain cases, the resultant level of population suppression can lead to eventual eradication of the target population. Furthermore, as SIT is inversely density-dependent, it becomes more effective when the wild population is low or reduced. The implementation of MAT and SIT has been sequential, with SIT applied after a significant reduction of the wild male insect population with MAT. This was to avoid the killing of released sterile males by semiochemical-baited MAT devices that targeted the male insects. We seek to explore the potentially synergistic relationship between MAT and SIT when applied simultaneously to improve the efficacy of Dacini fruit fly pest management.

Methods: Semiochemicals are used in MAT as an attractant to reduce the wild male population and can also be used as an enhancer to increase the sterile male mating performance in SIT. However, with the integration of MAT and SIT, high attraction of sterile males to MAT devices is undesirable and would significantly reduce the efficacy of SIT and increase the cost dramatically. Hence, various cost-effective semiochemical treatments and delivery systems that improve sterile male performance and reduce response to semiochemicals were explored for simultaneous MAT and SIT application.

Results: The combination of male replacement and enhanced male performance of sterile flies increases what can be achieved with the same number of sterile flies, thus, treating a wider area or enabling more rapid suppression or eradication. Due to the increased cost-effectiveness that results, decisions to invest in SIT may be facilitated in situations where this would not otherwise be feasible. Thus, more exploration of the potential of such approaches is essential given that the simultaneous

application of MAT and SIT has considerable potential to improve sterile to wild male over-flooding ratios, making SIT more cost-effective.

Conclusions: The incorporation of semiochemical treatments/supplements appears feasible and practical, however, standard procedures of implementation at an operational scale still need to be developed and validated.

Keywords:
Tephritid fruit flies, *Zeugodacus*, *Bactrocera*, SIT, MAT

Session 6: Sterile Insect Technique, November 17, 2022, 08:30 - 10:30

SITplus: Building a world-class sterile insect technique for Queensland fruit fly, *Bactrocera tryoni*

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Background: Sterile insect technique (SIT) is used worldwide to manage insect pests, including eradication of outbreaks and management of established populations. Successful SIT programs exist for several fruit flies, such as Mediterranean and Mexican fruit flies. While SIT had been used in Australia to control Queensland fruit fly (Qfly; *Bactrocera tryoni*), the program had not evolved beyond a basic level used to eradicate sporadic outbreaks. The SITplus program aims to take Qfly SIT to world-leading standards and become a standard management tool.

Methods: Hort Innovation, the grower-owned, not-for-profit research and development corporation for Australian horticulture, invested over AUD$60 million in SITplus to improve key processes of SIT for Qfly. Post-factory releases were undertaken to measure Qfly population suppression. Funding primarily came from external co-investment through research and state organisations, and matching contributions from the Australian government.

Results: Qfly SIT development has evolved rapidly. Changes to fly diet and rearing conditions reduced production costs while genetic research has produced new strains that will improve production outcomes. Post-factory seasonal release trials in New South Wales and Victoria saw significant reductions in wild Qfly populations.

Conclusion: This large program of work has greatly accelerated SIT for Qfly. The development of new strains provides major advances in the economic viability of Qfly SIT as well as assurances around successful SIT fly identification in traps. Finally, the seasonal release project showed that SIT successfully suppressed Qfly populations. The SITplus program has shown that collaboration and a sense of shared responsibility, Australian horticulture R&D levy-paying industries can reap long-term benefits by partnering with external collaborators.

Keywords: Sterile Insect Technique, Queensland fruit fly, *Bactrocera tryoni*

An efficient method to test screens for use in augmentoria applied to *Bactrocera oleae*, *Ceratitis capitata*, and two parasitoid wasp species

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Background: An augmentorium is a tent-like screened enclosure where infested fruits collected from the ground in an orchard or field can be deposited. The screen gauge retains any fruit flies that may emerge from these unusable fruits inside the tent but allows smaller parasitoid wasps that attack the fruit flies to exit. The goal is to enable field sanitation and removal of pest fruit flies while minimizing impact on beneficial parasitoids. Though the idea of an augmentorium against fruit flies has been in the literature for many years, implementation has been rare. One of the potential reasons for this is the lack of a simple method to test mesh screens for efficacy in particular applications.

Methods: We tested a variety of screen mesh types for use in augmentoria against the olive fly (*Bactrocera oleae*, Rossi) and Mediterranean fruit fly (*Ceratitis capitata*, Wiedmann) by creating "mini" augmentoria in the laboratory and introducing the two species of flies exposed to the parasitoids *Psyttalia lounsburyi* (Silvestri) and *P. ponerophaga* (Silvestri). For olive fly, infested olives were collected from the field and placed directly into the mini augmentoria for testing (50 olives per replicate, seven replicates). For Medfly, pupae resulting from colony larvae exposed to parasitoids in the lab were used (200 pupae per replicate, 7 replicates for *P. ponerophaga* and 5 for *P. lounsburyi*). We checked number and sex ratio of host flies and parasitoids retained and exiting each assay.

Results: We found a single mesh that was well suited to the goal, retaining 100% of the Medflies and 90% of the olive flies while allowing 90% of *P. lounsburyi* and 72% of the *P. ponerophaga* to escape. Sex ratios were generally the same for insects retained and escaping with this mesh.

Conclusion: In addition to finding a single promising mesh screen that may be effective for augmentorium implementation against both Medfly and olive fly we also demonstrated the utility of "mini" augmentoria in the laboratory to quickly assay screen types for different host pest/parasitoid combinations. Potential implementation details of the augmentorium approach for olive production remain to be determined.

Keywords: IPM, Sanitation, Braconidae, Agro-ecological management

Session 7: Natural Enemies and Biological Control, November 17, 2022, 11:00 - 13:00

Friend or foe: Symbiotic bacteria in *Bactrocera dorsalis*-parasitoid associations

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Background: *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), is a devastating fruit-infesting pest. Biological control using parasitoids is a promising avenue. However, the success of parasitoid-oriented biocontrol programs is a function of several factors, including the host-associated symbiotic-bacteria. *Providencia alcalifaciens*, *Citrobacter freundii* and *Lactococcus lactis* are among the symbiotic-bacteria commonly associated with *B. dorsalis* and were reported to alter its physio-ecological functioning. However, whether these bacteria influence the interaction between this pest and its parasitoids, is yet to be explored. In this regard, this study sought to elucidate the nature of the interaction of the three parasitoid species with *B. dorsalis* as mediated by symbiotic-bacteria.
Methods: The effect of symbiotic-bacteria (*P. alcalifaciens*, *C. freundii*, *L. lactis*) on parasitism success and fitness of parasitoid progeny of the egg parasitoid *Fopius arisanus* (Sonan), and the larval parasitoids *Diachasmimorpha longicaudata* (Ashmead) and *Psyttlia cosyrae* (Wilkinson) (all Hymenopteran: Braconidae) was investigated using B. dorsalis as a host., Fly-lines (from Dechorionated eggs) used in this study were: Axenic (bacteria free); symbiotic (intact microbiome); and bacteria mono-associated (either *P. alcalifaciens*, *C. freundii* or *L. lactis*), these were respectively reared on diets supplemented with distilled water, larval diet previously fed on by fruit flies with intact microbiome or with the individual bacteria. For the bacteria mono-associated fly-lines, was reared on their respective bacterial inoculum at 10^8 concentration. The suitable stages of each fly-lines were exposed to their respective parasitoid species. The parasitised host were reared till fly and/or parasitoid emergence. Thereafter, the number of emerged wasps, offspring developmental time, sex ratio, body size (in term of hind- tibia-length), fecundity and longevity were recorded.
Results: No wasps emerged from fly-lines exposed to *P. cosyrae*. For both *F. arisanus* and *D. longicaudata*-parasitised fly-lines, the highest emergence was recorded in the *L. lactis* fly-lines. Parasitoid developmental time was longest for *L. lactis* and *P. alcalifaciens* fly-lines. Parasitoid progeny eclosed from these fly-lines had largest body size. *Providencia alcalifaciens* enhanced parasitoid progeny fecundity whereas *L. lactis* negatively impacted the reproductive potential of the progeny of both parasitoid species, suggesting fitness costs associated with this bacterium. Surprisingly, there was no evidence of symbiont-mediated effects on the sex ratio and longevity of parasitoid progeny emerging from all fly-lines.

Conclusion: The results revealed a mixed nature of interaction being in general synergistic with parasitoid in case of *L. lactis* while it was more beneficial to the host flies in case of *P. alcalifaciens*, suggesting that symbiotic-bacteria could have significant consequences on host-parasitoid dynamics. We propose that *L. lactis* can be harnessed in mass rearing programs of *D. longicaudata* and *F. arisanus*.

Keywords: *Diachasmimorpha longicaudata*; *Lactococcus lactis*; *Fopius arisanus*; Tephritidae

Session 7: Natural Enemies and Biological Control, November 17, 2022, 11:00 - 13:00

Host-endoparasitoid-endosymbiont interactions: *Dipterophagus daci* (Strepsiptera) provides new twist to *Wolbachia* in Australian tephritids

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Background: The common insect endosymbiont *Wolbachia* affects arthropod reproduction and fitness, and is used in biological control. Mostly maternally inherited, *Wolbachia* is occasionally transferred horizontally within and between species. Previously, two *Wolbachia* strains were reported at low prevalence and loads in seven tephritid species (including Queensland fruit fly, *Bactrocera tryoni*) in tropical and subtropical Australia, possibly indicative of frequent horizontal transfer. However the origin of the strains and their interactions with tephritids remained unknown.

Methods: We used whole genome sequencing (WGS) to characterise *Wolbachia*-positive fruit flies. DNA extracts were obtained from male fruit flies collected across Queensland using male lure traps as part of fruit fly monitoring programs and PCR-screened for the previously characterised *Wolbachia* strains. Unexpectedly, the WGS data set contained complete mitogenomes of *Dipterophagus daci*, an understudied strepsipteran endoparasitoid that parasitises Australian tephritids. After its first genetic characterisation we developed PCR primers to specifically detect the mitochondrial *COI* gene of *D. daci*. Then we tested a large set of flies for presence of *Wolbachia* and *D. daci.* We also dissected stylopised flies to obtain neotenic females and male pupae of *D. daci*, and head, thorax and abdomen of the parasitised flies. DNA extracts of these samples were used to match *COI* sequences with the WGS data set, and to quantify *Wolbachia* load normalised to *D. daci* and fruit fly DNA.

Results: We detected *D. daci* in *Wolbachia*-positive flies which were not visibly parasitised. *Wolbachia*-negative flies were *D. daci*-negative, except for some individuals, possibly reflecting polymorphism for *Wolbachia* infections in *D. daci*. Therefore, *D. daci* was the likely true host of *Wolbachia* in flies. Using dissected *D. daci* from stylopised flies we confirmed that *Wolbachia* infects *D. daci*, but also found *Wolbachia* in stylopised fly tissues, likely somatic, horizontally transferred but non-heritable infections. The first phylogenetic analysis of *D. daci* confirmed its placement within Halictophagidae, and mitogenome analysis revealed low mitogenomic variation suggestive that *Wolbachia* plays a role in the strepsipteran's fitness, and extensive gene rearrangements as expected for endoparasitoids.

Conclusion: Our study highlights that *Wolbachia* detection in insects can be due to concealed parasitisation with *Wolbachia*-infected endoparasitoids and suggests that none of the 24 Australian tephritid species tested so far maintain heritable *Wolbachia*. Furthermore, using our diagnostic tools we found that up to 13% flies trapped in northern Queensland can be parasitised by *D. daci*, perhaps more than previously appreciated by assessing parasitisation rates based on stylopised flies. Therefore this unique strepsipteran may play an important role in fruit fly population dynamics where it occurs.

Keywords: *Bactrocera*; *Dacus*; *Zeugodacus*; *Wolbachia*; endoparasitoid

Session 7: Natural Enemies and Biological Control, November 17, 2022, 11:00 - 13:00

Modes and efficiency of transmission of *Iflavirus* and *Cripavirus* in Queensland fruit fly, *Bactrocera tryoni*

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Background: Insect-specific RNA viruses are common and can affect host fitness, health and immunity. Previously, several RNA viruses were identified in Queensland fruit fly (*Bactrocera tryoni*), Australia's most significant horticultural pest. Most prevalent were *Iflavirus* (*Iflaviridae*), *Cripavirus* (*Dicistroviridae*) and *Rhabdovirus* (Rhabdoviridae). The transmission mode and efficiency of these viruses are unknown, yet these factors may contribute to these viruses' epidemiology and the fitness of flies in field and laboratory populations. Our study investigated transmission of *Iflavirus* and *Cripavirus* in *B. tryoni*.

Methods: Individual pairs of virus-infected and uninfected flies were reciprocally crossed to assess horizontal transmission within mated pairs and vertical transmission from parents to offspring. Furthermore, virgin females of virus-infected and uninfected lines cohabiting in a cage were assessed for horizontal transmission; and bleached and unbleached embryos of infected females were assessed for vertical transmission. Viral load was determined using standard RT-PCR and RT-qPCR on pooled and individual RNA extracts converted into cDNA and were normalised against transcripts of the host gene elongation factor 1*a*.

Results: We detected three viruses (*Iflavirus*, *Cripavirus* and *Rhabdovirus*) in five *B. tryoni* laboratory populations recently established with field-collected flies in Queensland. Screening of individuals for viruses demonstrated their transmission is incomplete. Random segregation of viruses in an isofemale experiment resulted in the establishment of isofemale lines with and without *Iflavirus* and *Cripavirus* that enabled us to test their transmission. In infected lines, viral loads were found to be variable but generally lower in larvae than pupae or adults of both sexes. Both *Iflavirus* and *Cripavirus* were transmitted horizontally between cohabiting individuals, with viruses detected in many individuals after four days and most after 12 days cohabitation. *Cripavirus* was not detected in embryos, while *Iflavirus* was transmitted vertically, with surface-sterilised embryos containing high viral loads, and high viral loads in individual females resulted in high viral loads also in their offspring

Conclusion: The research findings about the transmission of viruses that are highly prevalent in laboratory populations is fundamentally important to establish and maintain uninfected fly lines and lines of known infection status for the assessment of virus host effects, as well as for application in pest management strategies such as the sterile insect technique (SIT) which requires the mass-rearing of flies whose fitness and performance may be affected by these viruses.

Keywords: *Bactrocera tryoni*, viral load, horizontal transmission, vertical transmission, *Picornavirales*

Releasing parasitoids for the control of *Bactrocera tryoni* (Froggatt) in Victoria

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Background: The withdrawal of organophosphate pesticides in recent years has driven a need for an effective toolkit of pest management practices to control Queensland fruit fly (*Bactrocera tryoni*), which has expanded its range into southern Australian states. Biocontrol using hymenopteran parasitoids has been utilised globally to help control tephritid pests but is lacking for *B. tryoni* in Australia. We conducted pilot releases of two parasitoids of Queensland fruit fly, *Fopius arisanus* and *Diachasmimorpha kraussii*, in Victoria; neither species having been recorded in the state at the beginning of the study. Evidence was sought for parasitism and within-season local establishment at release sites. We also assessed whether either parasitoid species can successfully parasitise the non-pest tephritid species *Urophora stylata* (gall thistle fly), a biocontrol agent against weed thistle species (*Cirsium vulgare*).

Methods: We released 95,000 parasitoids at three orchard and nine urban sites across Victoria in nineteen individual releases over two growing seasons. Within-season establishment of parasitoid populations at release sites was studied though the collection and rear-out of infested fruits at selected sites. We also conducted laboratory experiments to determine whether either species of wasp will parasitise immature *U. stylata*, using a no-choice experimental design that presented parasitoids with *U. stylata* infested thistle buds, and included positive and negative controls to confirm that parasitoids host finding behaviour and *U. stylata* oviposition occurred under the experimental conditions.

Results: Monitoring studies demonstrated mean parasitism immediately post-release as high as 58% for *F. arisanus* in a pome fruit orchard and 63% for *D. kraussii* in a garden environment, with average parasitism across all fruit collections more moderate at 8% and 17% for the two species respectively. *F. arisanus* populations persisted in the environment for at least 16 weeks post-release, and *D. kraussii* for seven weeks prior to winter 2022. Evidence for local dispersal of *D. kraussii* was found up to 1.5 km from one release site, approximately six weeks post release. Experiments evaluating parasitism of *U. stylata* by the parasitoids showed no evidence that either species will target or can parasitise this beneficial tephritid.

Conclusion: *Fopius arisanus* and *Diachasmimorpha kraussii* show potential for establishment in Victoria, persisting seasonally in the environment, reproducing, and dispersing from release sites. *D. kraussii* is perhaps the best candidate for further research on augmentative releases, due to ease of mass rearing, greater overall establishment at release sites, as well as a recent identification of a small population of this species in NE Victoria. Neither species of parasitoid will impact *U. stylata* populations and the biocontrol of thistles.

Keywords: Queensland Fruit Fly, *Bactrocera tryoni*, *Fopius arisanus*, *Diachasmimorpha kraussii*, Parasitoid, Integrated Pest Management, *Urophora stylata*

Session 7: Natural Enemies and Biological Control, November 17, 2022, 11:00 - 13:00

The dual applications of entomopathogenic fungus and methyl eugenol for controlling *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) under field condition in Thailand

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Background: The Oriental fruit fly, *Bactrocera dorsalis*, is one of the most destructive pests of agricultural crops in Thailand. *B. dorsalis* is a highly polyphagous insect attack many commercially cultivated horticultural crops with up to 100% loss to unprotected fruits caused both economic losses at field and strict quarantine restrictions on export. Entomopathogenic fungus, *Metarhizium quizhouense*, are being tested as potential biocontrol agents to control fruit flies and methyl eugenol is a powerful semiochemical attractant to male of *B. dorsalis*. The present study, we evaluated the dual applications of *M. guizhouense* PSUM04 conidia in vegetable oil-based formulation and methyl eugenol in rubber foam technology with designing of an attractant-inoculation auto-dissemination device on infection of adult male *B. dorsalis* and long term horizontal transmission in the wild population under field conditions.

Methods: The field experiment was conducted in the three sapodilla orchards located in Koh Yor, Songkhla province, Thailand. The 1st orchard, the attractant inoculation device (AID) was replaced monthly. The 2nd orchard, the AID was not replaced. The 3rd orchard was set as the control. Twenty AID were installed in each orchard. The AID were made from 1.25 litter clear plastic bottles with two sides of 20 cm holes, one on each side, were made in the middle of a clear plastic bottle for entrance and exit of adult male *B. dorsalis*. Methyl eugenol in the rubber foam was set inside the traps as an attractant and covered with impregnated *M. guizhouense* PSUM04 conidia (1×10^{10} spore/ml) terry cloth in soybean oil based formulation (1:9). Fruit fly populations were monitored using twelve 500 ml plastic cup traps with methyl eugenol in rubber foam and 20% propylene glycol was added to collect and preserve attracted flies. Another five plastic cup traps, without propylene glycol, were used to collect flies in order to check the percentage of infections. The number of attracted flies in all traps and the percentage infections in each orchard were recorded every two weeks for six months.

Results: Efficiency of AID replaced after 30 days under field conditions showed the highest reduction in the field population of *B. dorsalis* along with most infected males and females. The horizontal transmission of fungal pathogen was found sufficient to cause infection in wild flies in the first eight weeks of experiment due to humid climatic conditions.

Conclusion: The integration of *M. guizhouense* PSUM04 in an oil formulation based as an attractant-inoculation device (AID) could help in the suppression of wild population of *B. dorsalis* under field conditions with area wide management programme.

Keywords: *Metarhizium guizhouense*, vegetable oil-based formulation, rubber foam lure, attractant-inoculation device, horizontal transmission

Session 8: Other Control Methods or New Developments, November 17, 2022, 14:30 - 16:30

A holistic approach to address invasive fruit flies (Diptera: Tephritidae) in Europe: the FF-IPM project

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Background: Fruit fly invasions threaten the fresh fruit production and trade worldwide. This threat is enormous in Europe with several, mainly tropical, species intercepted in ports of entry and, currently, detected in "friendly" for their establishment areas. In addition, the Mediterranean fruit fly, *Ceratitis capitata,* in response to climate change, is progressively dispersing to more northern latitudes. The EU funded project FF-IPM "In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies" targets two invasive (i.e. *Bactrocera dorsalis, B. zonata*) and one emerging (*C. capitata*) pests addressing all steps of invasion.
Methods: Extensive laboratory and field cage experiments using wild populations, release recaptures studies in open field conditions, review of the existing literature, and pilot testing in several countries have been conducted. Several functions to automatically collect meteorological data have been developed to feed population modelling approaches. From the study design to implementation and pilot testing the active involvement of stakeholders have been assured. Twenty one partners of the FF-IPM project have been involved in experimental and other activities throughout 10 European and four non-European countries.
Results: Novel data, including thermal biology, dispersion capacity, establishment dynamics and genetic affinity of wild populations, of the above species s have been generated. Advanced climatic modeling using the CLIMEX and DYMEX platforms have been generated. Advanced interception and detection tools have been developed, like an e-nose system to intercept fruit fly infested fruit, molecular and morphological identification tools, e- traps to detect and algorithms to automatically recognize and count captured adults. A range of Integrated Pest Management (IPM) tools have been developed, improved, and tested for Off-Season management, including mass trapping devices, entomopathogenic nematodes and fungi, methodology for increasing the abundance and activity of ground predators. Innovative approaches to assess the risk of fruit fly establishment have been developed and new strategies to increase detection efficacy are tested in pilot sites in four countries. Off an On-Season IPM approaches based on the PestOnFarm platform to address *C. capitata* in areas considered marginal for its establishment have been developed and are tested in six pilot sites in three countries.
Conclusion: The FF-IPM project has generated important results, tools and methods to address invasive fruit flies in Europe at all steps of the invasion process. The problem of fruit fly invasion is multidimensional, complex and requires involvement of all stakeholders as well as coordination and support by national, European and regional organizations. We discuss the weaknesses of the European system to deal with ongoing invasion events and the opportunities to establish a more efficient preparedness strategy against invasions in fruit producing systems.

Keywords: Biological invasions, Oriental fruit fly, Peach fruit fly, thermal physiology, trapping, interception, detection

Session 8: Other Control Methods or New Developments, November 17, 2022, 14:30 - 16:30

A systems approach for management of fruit fly pests in citrus in South Africa

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Background: South Africa is among the largest exporters of citrus in the world. Fruit flies pose a serious threat to this industry as there is a zero tolerance of fruit flies in exported South African citrus. Three fruit fly species are listed as citrus pests in the country. These are *Ceratitis capitata* (Wiedemann), *Ceratitis rosa* Karsch and *Bactrocera dorsalis* (Hendel). In order to mitigate the risks of these species in citrus exported from South Africa, single point cold disinfestation treatments at extreme temperatures (mostly at temperatures below 0°C for 22 days) have been required by some markets. Temperatures below 3°C are detrimental to the quality of some citrus types, limiting the feasibility of this treatment. The fruit fly systems approach was developed as an alternative to single point cold disinfestation treatments for citrus from South Africa, combining pre-harvest and post-harvest control measures. In order to determine the efficacy of the systems approach, the efficacy of each key control measure was quantified: (1) fruit fly control in orchards; (2) packhouse sorting and grading; and (3) post-harvest cold treatments at temperatures above 3°C.

Methods: (1) Field trials were conducted in citrus orchards to evaluate the efficacy of protein baits in reducing fruit fly populations and fruit infestation. (2) In a study conducted between 2020 and 2022 in 26 commercial citrus orchards, fruit were sampled before harvest, after harvest and after packhouse sorting and grading to determine fruit fly infestation level. (3) The most cold tolerant fruit fly species was determined, and cold treatments were conducted using this species to determine mortality following exposure to temperatures at 3.5°C for 24 days and at 5°C for 27 days.

Results: (1) Protein baits applied in citrus orchards kept fruit fly population levels low and limited fruit fly infestation. (2) In the 2020-2022 study, fruit infestation in commercial citrus orchards was low (less than 0.5%). Packhouse treatments further reduced fruit fly infestation. *Ceratitis capitata* was the only species reared from infested citrus. (3) *Ceratitis capitata* was found to be more cold tolerant than *C. rosa* and *B. dorsalis*. There were no survivors of *C. capitata* following cold treatments at 3.5°C for 24 days and 5°C for 27 days.

Conclusion: A fruit fly systems approach combining (1) fruit fly control measures in commercial citrus orchards prior to harvest, (2) packhouse measures to remove potentially infested fruit in the packing line and (3) cold treatments at pulp temperatures of either 3.5°C for 24 days or 5°C for 27 days should effectively mitigate the risk of fruit flies in export citrus from South Africa.

Keywords: systems approach, *Ceratitis capitata*, citrus

Session 8: Other Control Methods or New Developments, November 17, 2022, 14:30 - 16:30

Assessing attraction and toxicity of protein bait products to fruit flies

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Background:
The Queensland fruit fly (Q-fly), *Bactrocera tryoni* (Froggatt), is the main endemic pest fruit fly in Australia but is considered absent from South Australia, Western Australia, and Tasmania. The Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann), is invasive to Western Australia. The control of fruit fly invasions in pest-free areas is expensive and strongly dependent on the accessibility of a limited set of tools. The uptake of new tools in governmental response programs is often hampered by the availability of independent comparative data against a 'traditional' baseline. The aim of this study was to develop bioassay methods to obtain statistically powerful datasets to compare commercial products against a baseline product in a short timeframe.

Methods:
To assess both attraction and toxicity of fresh and aged products, a series of three bioassays measuring fly behavioural response to attractant stimuli and mortality under controlled laboratory conditions and in field cages was developed. The target species (Q-fly and medfly) and assessed products varied between trials. Toxicity was tested by exposing small cohorts of flies to treated substrates in small plastic containers and measuring fly mortality. Both attraction and toxicity of the products to flies was evaluated in wind tunnels by scoring fly visitation to baits, and subsequent mortality. Field cage trials were conducted to verify the results under more realistic conditions.

Results:
Bioassays generated powerful data in a short time, that could detect fine differences in fly behavioural and mortality responses to different fresh and aged bait products. A preliminary data analysis found that alternative products caused comparable rates of fly mortality to the NaturaLure® standard when fresh, but clear differences between products emerged after periods of ageing. In addition, the time to death after the initial exposure differed between products. The results inform product selection and the likely relative persistence of different products after field application.

Conclusion:
We found that alternative products have the potential to attract and kill flies at comparable rates to established products. The bioassay method worked well with both medlfy and Q-fly and present a valuable tool to directly compare any alternative product mixes in the future. Field experiments, which are typically more costly and less statistically powerful due to higher variability in the data, can then be used efficiently as a tool to confirm expected results.

Keywords: Queensland fruit fly, Mediterranean fruit fly, pest management, protein baits

Daily Accurate Fruit Fly Forecast Enabled by Internet of Things Sensors

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Technological advances in automated pest detection are enabling high quality pest information. The commercial RapidAIM - RapidFLY service that provides real-time detection for Queensland fruit fly (Qfly), *Bactocera tryoni*, is one such example. Real-time Qfly detections are enabled by the fully integrated Internet of Things (IoT) insect sensors (www.rapidaim.io). These RapidFLY IoT sensors are deployed by customers; > 700 sensors across the eastern states of Australia. The real-time Qfly data flowing from the RapidFLY IoT sensors can be aggregated across regions to deliver high quality information for more efficient pest management.

Here we showcase the *Regional Daily Fruit Fly Forecast* where the data is ingested into models for a broad range of pest analytics. Using artificial intelligence and machine learning, forecasts are generated across regions of 500 – 700 km^2. The high-quality, highly granular data flow from the IoT sensors results in model fit > 85% for daily five-day Qfly forecast. Qfly persistence, regional coverage, peaks, seasonal bench marking, historical trends, weather and daily model fit is some of the information provided from the commercial service.

The information from the service is used by crop advisors and growers to plan and prepare for the week, whereas the RapidFLY on-farm grid of sensors is used by growers to know when and where fruit fly shows up on their property and for them to validate whether control is working. Queensland fruit fly is the first pest for the commercial service of regional daily accurate pest forecast, with broad application to more pests.

Insight on Fruit Fly IPM Technology Uptake and Barriers to Scaling in Africa

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Background: Tephritid fruit flies are considered one of the world's most notorious pests of horticultural crops, causing extensive direct and indirect damage. Over the past two decades, a comprehensive, integrated pest management (IPM) package for the management of a plethora of fruit fly pests, including *Bactrocera dorsalis*, *B. latifrons*, *B. zonata*, *Ceratitis cosyra*, *C. rosa*, *C. fasciventris*, *C. quilici*, *C. capitata*, *Dacus* spp. and *Zeugodacus cucurbitae*, has been developed, disseminated and promoted among horticultural growers in Africa. Therefore, the fruit fly IPM technology uptake, number of beneficiaries and barriers to scaling in Africa were new areas of research.

Methods: We estimated the numbers of beneficiaries reached by the fruit fly IPM technology and the barriers to technology uptake by interviewing 290 experts in 30 African countries covering five regions of the continent, and the responses collected were represented as follows: Southern Africa (39.1%), Eastern Africa (31.6%), Western Africa (18.0%), Central Africa (9.0%) and Northern Africa (2.0%).

Results: Our results revealed that the use of fruit fly IPM technologies varied across the regions, with Eastern Africa and Western Africa the leading regions, with the highest IPM technology penetration. Field sanitation remains the most common practice for managing fruit flies, followed by protein bait spray, use of biopesticides, male annihilation technique and parasitoid releases. According to the survey, over 101 million people have benefited from the fruit fly IPM interventions in the countries surveyed representing only 19.1% of the estimated beneficiaries. The region that benefitted the most was Eastern Africa (50.2 million), followed by Central and Western Africa (11.7 to 17.7 million), and Southern and Northern Africa had the fewest beneficiaries (10.4 to 11.0 million). The limitations to the IPM technologies uptake varied among the regions, but the common ones include a lack of awareness of the IPM technologies, a lack of access to the IPM products, insufficient training, low involvement of private sectors and a lack of policies for the regulation of IPM technologies.

Conclusion: Although significant strides have been made in promoting the fruit fly IPM technologies over the past two decades, our study reveals that the demand surpasses the current supply. Therefore, we recommend a comprehensive strategy for the dissemination and promotion of the technologies through a multi-institutional alliance that enhances public and private partnerships, digital platforms and youth engagement to consolidate previous gains at the regional and continental levels.

Key words: Beneficiaries, Fruit flies, IPM technologies, Horticulture

Session 8: Other Control Methods or New Developments, November 17, 2022, 14:30 - 16:30

Smart-Traps for Fruit Flies: Their Integration into Pest Management and Biosecurity

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Background: Smart-traps for fruit flies (i.e., traps aimed for real-time and remote monitoring of adult fruit flies) are being proposed and developed at a fast rate by many groups, and their applications in fruit fly pest management and surveillance is becoming a reality. Smart-traps are constituted by an electronic and communication system, and detect, count and classify fruit flies with the assistance of electronic sensors, such as optical, electromagnetic and acoustic. In general, most smart-traps proposed and developed for fruit flies utilize optical sensor, either to count flies through photo-interruption, or by obtaining high-resolution images that can be further analysed with image processing techniques. In addition, some currently commercialized smart-trap sense entering fruit flies into the trap with electrical sensors sustained by capacitors. During the last years, some of these smart-trap prototypes have been tested and used in fruit fly pest management research. More recently, some commercial companies are already providing fruit fly management services using smart-traps as a source of information on fruit fly pest levels in space and time for decision-making.

Methods: In our laboratory, we developed two proto-types of optical smart-traps for fruit flies:
(1) A yellow sticky-board e-trap conceived as a "universal" device for three species of fruit flies (*Bactrocera olea*, *Rhagoletis cerasi* and *Dacus ciliatus*), and based on their attraction to colour (yellow board)
(2) A McPhail E-trap that is combined with parapheromone attractants and aimed for surveillance of *Bactrocera* and *Ceratitis* species.
Their performance was, and is being, investigated under two projects: The accomplished *FruitFly Net* project and the ongoing *FF-IPM* (both supported by the EU). Besides testing the reliability of the traps and contrast them to conventional traps used to attract these fruit flies, we also tested their use for decision-making in extensive experiments under field conditions.

Results: The yellow-sticky-board e-trap prototypes provided good monitoring results with the three species of flies and environments, and the information served to implement and test developed Decision Support Systems (DSS) algorithms. In general, DSS provided good control of damage. The McPhail e-trap is currently being implemented and tested as part of a surveillance strategy for invasive and expanding fruit flies. Preliminary results suggest that they provide good early-warning alerts.

Conclusion: Smart-traps are proving to be valuable tools for fruit fly pest management. Their recent commercialization and integration into pest management is a proof of their value. It is expected that more effort and devices will be developed in the future, targeting and complementing other fruit fly management tools, such as SIT.

Keywords: electronic insect traps, *Bactrocera*, *Ceratitis*, Early Warning

Session 9: Area-Wide IPM and Action Programs, November 18, 2022, 08:30 - 10:30

A multifaceted approach to domestic management of invasive fruit fly threat in the United States

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Background: Tephritid fruit flies are among the most destructive pests to fruit and vegetable crops around the globe and are some of the pests of greatest phytosanitary concern to the United States. The United States federal government, in collaboration with international, state, tribal, and local governments, has a long-standing, national fruit fly program focused on protecting U.S. agriculture by preventing the entry, establishment, and spread of exotic fruit flies to the United States.
Methods: We analyzed the United States' multifaceted strategy for managing multiple fruit fly species simultaneously by focusing on three main fruit fly quarantine pests: Mediterranean fruit fly (Medfly, *Ceratitis capitata*), Mexican fruit fly (Mexfly, *Anastrepha ludens*), and Oriental fruit fly (OFF, *Bactrocera dorsalis*). We compiled, compared, and contrasted publicly available program documents guiding the management of these pests to identify similarities and differences in management strategies among them.
Results: While there are general principles that are applied throughout the United States' fruit fly program, specific program activities differ among target species based on their biology, susceptible hosts, available attractants, detection tools, and treatments. To prevent infestation, the United States enacts year-round preventive sterile insect release programs to target two fruit flies (Medfly and Mexfly) in three states (California, Florida, and Texas). This requires production of fruit flies in four mass-rearing facilities in two states (Hawaii and Texas) and one foreign country (Guatemala), and six eclosion and release facilities in three states (California, Florida, and Texas) and one foreign country (Mexico). The United States also deploys an extensive trapping network across ten states with suitable hosts and climatic conditions for exotic fruit fly establishment. When an exotic fruit fly is detected in any state, an emergency response is triggered based on the identity of the fly and the number of adjacent flies detected within a single life cycle. The more difficult to detect and treat the fly species, the fewer detections are required to trigger an emergency response. Emergency responses are staggered from delimitation to eradication to quarantine, with each successive step requiring a greater degree of trapping, treatments, and regulations, depending on the specific situation.
Conclusion: This complex system is managed in an integrated approach through agreements between federal, state, and local governments, and requires a high degree of oversight from the central governing body within the USDA to ensure standardization of operations and fair distribution of funds according to relative need and fruit fly threat. Among other responsibilities, this body also seeks out and promotes scientific research that can help to introduce improved efficiencies into current fruit fly operations.

Keywords: detection trapping, emergency response, Mediterranean fruit fly, Mexican fruit fly, Oriental fruit fly, SIT

Session 9: Area-Wide IPM and Action Programs, November 18, 2022, 08:30 - 10:30

Applying Lessons of the Past to Recovery Strategy for Medfly in Guatemala

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Background: Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), or Medfly is an economically and environmentally significant agricultural pest due to the scale of damage it causes to fresh fruits and vegetables within its extensive host range. Introduced into South and Central America in 1901 in Brazil and 1955 in Costa Rica, Medfly expanded quickly, reaching Guatemala in 1975 and Mexico in 1977.
Through a series of bilateral agreements and now a trilateral, the United States, Mexico, and Guatemala cooperate on the Moscamed Program. In Guatemala, Moscamed, advised by the trilateral entities, implements an integrated pest management (IPM) program with a sterile insect technique (SIT) component. Moscamed provides direction from a central office to two sterile fruit fly mass production facilities (one *Ceratitis capitata* and the other *Anastrepha ludens*) rearing strains with traits to improve efficiency and efficacy; a sterile Medfly emergence facility and release center; and field offices conducting surveillance and terrestrial control activities.
Medfly seasonality in Guatemala translates to higher captures and dispersion in quarter (Q) 2 and Q3 of the calendar year, and lower captures in Q1 and Q4 as populations recede into source reservoirs. In 2015, Moscamed effectively managed Medfly, with wild captures in 12% of the working area during the high season. However, in 2019, due to climatic conditions and reductions in control activities, Medfly populations increased significantly, with wild captures in 52% of the working area.
Methods: Moscamed tailored technical responses to hosts, seasonality and social challenges. Medfly in the northeast was addressed with a conventional IPM strategy, including enhanced detection, host removal, terrestrial treatment with an organic pest-specific insecticide, and SIT via ground release. In the southwest, where a long, dense coffee belt is a conduit for rapid pest population growth and northward movement, Moscamed conducted targeted aerial applications of organic insecticide in Q1 and Q4, focusing on reservoir populations. The northwest offers more dispersed hosts and access challenges preventing execution of Medfly-related activities. In this area, Moscamed enhanced outreach to gain access for Medfly surveillance, ground controls and SIT, implementing a rolling carpet strategy for recovering area near the border with Mexico.
Results: The treatment schemes effectively reduced pest populations to levels manageable with SIT. Timing enhanced control activities during Q1 and Q4 of 2020 and 2021, combined with IPM strategies, reduced wild Medfly captures to 20% of the working area in Q1 2022.
Conclusion: Moscamed regained control of the pest through deployment of a targeted technical approach, including high quality sterile Medfly; well-timed aerial and ground chemical control application and innovative wax bait stations; and outreach.

Keywords: Mediterranean fruit fly, Integrated Pest Management (IPM), Sterile Insect Technique (SIT)

Session 9: Area-Wide IPM and Action Programs, November 18, 2022, 08:30 - 10:30

Area-wide management of Queensland fruit flies in Victoria's Goulburn Murray Valley by a combined government, industry, community and science approach

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Background:Goulburn Murray Valley (GMV) is a horticultural region in Victoria mainly growing stone and pome fruit. Queensland fruit fly established in the region in 2013. In 2017 the Goulburn Murray Valley fruit fly Area Wide Management program was established to build community awareness and ownership of Queensland fruit fly management in the region and to suppress fruit fly populations. A key to achieving these aims is the involvement of a range of regional stakeholders including community groups, local and state governments and local horticulture industry associations.
Methods: The GMV Governance Group, representing Agriculture Victoria, local councils, horticultural industries/grower organisations, community groups and scientists developed area-wide management (AWM) action/implementation plans. The govenance group members and the coordinator made a case for participation in delivery of the plan to a range of stakeholders through meetings and presentations to outline how their contribution could assist the regional to improve the management of Queensland fruit flies. Queensland fruit flies were monitored weekly during the fruit growing season and fortnightly in the winter.
Results: This initiative resulted in the GMV fruit fly program achieving ongoing volunteer support and ownership which led to broad community engagement across the entire region.
Activities by approximately 3000 volunteers included - assisting with the installation of 501 road signs to build awareness of fruit flies, workshops for producers and the community, field days and a school-based education program which in total reached 16,954 people and distributed 53,996 information packs. State and local governments provided points of contact for the community linking them to fruit fly information and voluntary tree removal programs. Tree removals by GMV local governments removed 22,285 trees from home gardens and public land and 81,662 trees from unmanaged orchards. Industry groups participated in media campaigns to encourage the community to act to manage fruit fly. This complemented the other work by the program such as fruit fly monitoring, host plant removals, monthly fruit fly situation updates, mass-media awareness, education and support for the SIT pilot in the GMV.
The program achieved broad-based community engagement about fruit flies which would not have been possible without the involvement of all regional stakeholders. There has been a reduction in the fruit fly numbers over the four years of the program compared to the previous four years.
Conclusion: AWM programs require the support of diverse stakeholders to engage with the community and promote change. Engagement is likely to be more successful with a whole-of-community approach based on the active involvement of government, industry, community and the use of science-based decision making.

Keywords: Queensland fruit fly, Area-wide management, whole-of-community approach

Session 9: Area-Wide IPM and Action Programs, November 18, 2022, 08:30 - 10:30

Eradicating Queensland fruit fly (*Bactrocera tryoni*) in Perth Western Australia – history, challenges, and future directions

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Background: In Australia, the main fruit fly species of economic concern are Queensland fruit fly, QFF (*Bactrocera tryoni*) and Mediterranean fruit fly, MFF (*Ceratitis capitata*). Both species have restricted distributions, with QFF found in the eastern states but absent from Tasmania, South Australia, and Western Australia (WA). MFF has a limited distribution within WA and is not established elsewhere in Australia.

Methods: An early warning surveillance (trapping) array for QFF (cue-lure) and exotic fruit fly species (methyl eugenol, capilure) is maintained in WA. WA also maintains area freedom in the Ord River Irrigation Area from MFF. Traps are deployed at the rate of 1/400m^2 within urban areas and 1 km^2 in commercial horticultural production areas. When trap catches exceed a nationally and internationally agreed threshold, an eradication program is executed.

Results: WA has responded to seven QFF incursions, all within the Perth metropolitan area where MFF is established. The first eradication effort in 1989 covered 30,000 hectares and cost A$8 million (A$17.8 million adjusted for inflation). Techniques included protein baiting with malathion, male annihilation (cue lure/malathion in caneite blocks), and release of sterile QFF reared in a purpose-built WA facility. Data was recorded on Microsoft Excel spreadsheets and larval rear out was used to distinguish between MFF and QFF. Subsequent outbreaks in 1995, 2015 and 2018 were eradicated using protein baiting (Spinosad in Naturalure® Fruit Fly Concentrate) alone.

The 2020 response cost A$14.26 million to eradicate QFF from 2,053 hectares and was challenging because of the Covid-19 pandemic. Additional techniques included mass trapping (BioTrap gel traps), deployment of commercially produced Amulet® Cue Lure stations (cardboard wafer), and release of sterile QFF obtained from the SIT Plus facility in South Australia. The identification of larvae in fruit using molecular techniques enabled rapid identification of QFF breeding sites and evaluation of the efficacy of protein baits using MFF as the indicator species. Data was recorded in MAX which runs on Microsoft SharePoint software.

Conclusion: Eradication of QFF over the last 20 years has becoming more challenging for several reasons including increasing urbanisation, problems in resourcing sufficient staff for short term responses, and reduction in the availability of insecticides suitable for use in urban and public areas. However, advances in molecular identification of larvae in fruit and the use of geographical information software has enabled improvements to be made.

Keywords: Queensland fruit fly, eradication

Eradication of the Mediterranean Fruit Fly, Ceratitis capitata (Wiedemann) in Colima, México

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Background: In order to detect in a timely manner any entry of more than 30 species of exotic fruit flies of the genera Ceratitis, Dacus, Bactrocera and some species of Anastrepha and Rhagoletis, the National Service for Agrifood Health, Safety and Quality of Mexico operates a National Phytosanitary Epidemiological Surveillance System, consisting of around 16,000 traps located in the riskiest sites, such as ports, airports, migratory paths and production areas. On April 6, 2021, the Mediterranean fruit fly, Ceratitis capitata (Wiedemann) was detected in the Port of Manzanillo, Colima.

Methods: Derived from this detection, a National Emergency Device was implemented. Seven verification and inspection points were installed in the main highways. A detection and monitoring net was established using traps baited with specific attractants and fruit sampling. Phytosanitary eradication measures, based on an integrated pest management approach were implemented, including bait stations with hydrolyzed protein, mass trapping of males, destruction of host fruits, bait sprays with an organic product based on Spinosad, ground releases of the larval parasitoid Diachasmimorpha longicaudata and aerial and ground release of sterile flies. All phytosanitary measures were accompanied by outreach activities to raise public awareness and the technicalscientific support of national and international experts.

Results: The Mediterranean fly was recorded in 169 km2 of the urban area, affecting tropical almond fruits (Terminalia catappa, L.) mainly, in a regulated area of 4,289 km^2. After 10 cycles of absence of the pest and in compliance with the International Standard for Phytosanitary Measures No. 8, the Mediterranean fruti fly was considered eradicated from the municipalities of Armería, Colima, Comala, Coquimatlán, Ixtlahuacán, Manzanillo, Tecomán and Villa de Álvarez, in the state of Colima, Mexico, and the current phytosanitary status is pest absent. This was notified in the web site of the North American Plant Protection Organization (NAPPO) on August 2, 2022.

Conclusion: The Mediterranean fruit fly program in Mexico has the technological package to achieve eradication of this important pest.

Keywords: Mediterranean fruit fly, eradication, sterile insect technique, area-wide pest management program

Fruit fly control in Mauritius

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Background: Fruit flies (Diptera: Tephritidae) are among the most destructive and widespread pests of fruits and vegetables in Mauritius. This situation is emphasized under insular and tropical conditions. Fruit flies of economic importance are *Bactrocera dorsalis* (Hendel), *B. zonata* (Saunders), *Ceratitis capitata* (Wiedemann), *C. quilicii* De Meyer, Mwatawala et Virgilio that cause damage to fruits (mango, guava, jujube and peach among others), *Zeugodacus cucurbitae* (Coquillett), *Dacus ciliatus* Loew and *D. demmerezi* (Bezzi) are pests of cucurbits (cucumber, pumpkin and squash among others). *Carpomya vesuviana* Costa attacks only the ber fruit while *Neoceratitis cyanescens* (Bezzi) is a pest of tomato. Planters tend to make heavy use of pesticides to control fruit flies. However, this method of control is not sustainable and poses a threat to both the environment and health. An integrated fruit fly control programme with environment friendly techniques is being implemented in planters' fields.

Methods: The bait application technique and the male annihilation technique have been used to suppress the fruit fly population in orchards and cucurbit plantations in selected regions. Waste brewery yeast collected from the local brewery was modified into fruit fly bait for use in bait sprays at the rate of 10% v/v mixed with an insecticide. Bait sprays were applied at weekly intervals along border crops in cucurbit plantations while all trees in the orchards were treated. Plywood blocks impregnated with either cuelure or methyl eugenol mixed with Malathion ULV were placed on trees for mass trapping of male fruit flies. Pilot ground releases of sterile *B. dorsalis, B. zonata* and *Z. cucurbitae* using the paper bags were also carried out. Fruit fly traps lured with the pheromones were used to monitor the fruit fly population. Larval surveillance was also carried out.

Results: The effectiveness of areawide fruit fly control measures in the targeted cucurbit plantations and fruit trees in backyards was demonstrated by a reduction of larval infestation in both wild and cultivated fruits, and by a simultaneous decrease in fruit fly trap catches. The control programme was effective in maintaining significantly lower fruit fly population levels in all treated areas. Farmers used fewer pesticides while cucurbit infestation by fruit flies was reduced from >30% to <5%.

Conclusion: Larval surveillance data showed that *B. dorsalis* has displaced *B. zonata, C. quilicii* and *C. capitata* in mango, carambola, guava, Indian almond, and citrus. However, *Z. cucurbitae* remained the main fruit fly species that attacked the cucurbits. Results showed that fruit flies can be successfully suppressed by areawide application of protein bait sprays, mass trapping of males and release of sterile flies.

Keywords: Sterile insect technique, Oriental fruit fly, Peach fruit fly, Melon fly

Session 10: Social, Economic and Policy Issues of Action Programs, November 18, 2022, 11:00 - 13:00

Collaboration: key to a strong and robust national fruit fly management system.

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Background:

Australia has a plant production system, including agriculture and forestry, worth $25 billion annually and a unique environment to protect. The system not only supports the livelihoods and investments of individual producers, it also protects consumers in domestic and export markets, by maintaining the integrity, quality and sustainability of Australia's food supply.

Australia's National Plant Biosecurity Strategy (2021-31) provides a blueprint for effective collaboration and action by all participants, including governments, plant industries and the community leading to a resilient and contemporary system that supports our plant industries, economy, environment, and communities.

In Australia's national context, collaboration plays a critical role in ensuring our biosecurity systems are robust, consistent and provide assurance to both external and internal stakeholders. The fruit fly system is no exception.

Methods:

Our National Fruit Fly Strategy (NFFS) has provided a framework to ensure we effectively manage fruit flies. Gaining and maintaining access to premium markets for Australia's horticultural products relies on cooperation at all levels of government, and between industry bodies, research institutions, regional groups, growers, home gardeners, and the community in general.

The complex process of national coordination of fruit fly management is delivered through the broad strategic framework for plant biosecurity, with an overarching Intergovernmental Agreement on Biosecurity (IGAB). The National Fruit Fly Strategy 2020-25 stems from this agreement, with oversight for its implementation led by the National Fruit Fly Council (NFFC). The NFFC provides strategic advice and leadership on fruit fly policy and RD&E more broadly including to the National Biosecurity Committee, Plant Health Committee, Hort Innovation, industry, and the community.

The NFFS has a simple vision; 'Ensure Australia has a robust fruit fly management system that supports growth in horticultural production, market access and trade'. This vision is delivered through a set of eight key priorities including market access; management of established fruit fly; prevention, preparedness and response; research; surveillance; diagnostics; communication and engagement; and cooperation. A key focus of the Council is further development of cooperation across the national system and the establishment of a sustainable funding model to deliver contemporary solutions both now and into the future.

Results:

Increased collaboration across all facets of the national fruit fly ecosystem has enabled strengthened partnerships and enhanced two-way communication with deep engagement ensuring we achieve our vision of growth in market access and trade. This, coupled with a long-term sustainable funding stream will do much to future proof the system.

Conclusion:

A strong and robust national fruit fly management system ultimately relies on a clear and coherent strategy that is delivered through a range of industry, government, research and community settings that are intimately connected and adequately resourced.

Session 10: Social, Economic and Policy Issues of Action Programs, November 18, 2022, 11:00 - 13:00

Fruit Fly (Diptera: Tephritidae) International Standards for Phytosanitary Measures Can Facilitate Safe Trade

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Background: Fruit flies (Diptera: Tephritid) present a major challenge to international trade of fruit and vegetables, because if they are detected in fruits or vegetables, trade may be stopped. To facilitate the trade, between 2006 and 2016 a set of fruit fly International Standards for Phytosanitary Measures (ISPMs) was adopted by the Commission on Phytosanitary Measures (CPM), the governing body of International Plant Protection Convention (IPPC). Those standards were developed by IPPC Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies (TPFF) and follow the IPPC approval and adoption process. In 2017 a reorganization of the fruit fly ISPMs was initiated resulting in the actual set of standards available to the countries to implement with the objective to facilitate fruits and vegetables trade.
Methods: The set of ISPMs on fruit flies has been developed and recently reorganized. Those include: ISPM 26 (Establishment of pest free areas for fruit flies (Tephritidae)); ISPM 35 (Systems approach for pest risk management of fruit flies (Tephritidae)) and ISPM37 (Determination of host status of fruits to fruit flies (Tephritidae)) with nine additional annexes and appendixes.
Results: When a country wants to export fruit, this set of standards can facilitate the process. It starts with the ISPM37 for "host status" which questions: Can this fruit be a host for fruit flies? If it is a non-host and therefore it can proceed directly for export. But if there is a possibility that the pest infests the fruit, then the question is whether the fruit comes from an area that is fruit-fly free (ISPM 26). If it does, then it can go for export. But if fruit flies are present in this area, then the country must apply additional phytosanitary measures to reduce the risk of fruit fly hosts introducing the pest to the importing country. This scheme is called systems approach (ISPM 35). In a systems approach, the exporting country applies two or more independent phytosanitary measures. The integration of these measures will reduce the risk of pests in fruit and vegetable trade to the levels that are acceptable by the importing country, while contributing to assuring high quality of the fruits to be exported. The phytosanitary scheme under which trade might occur is defined and agreed by the interested parties, based on ISPMs or technically justified. This is done through a bilateral agreement between the plant protection organizations of both exporting and the importing countries.
Conclusions: Through the implementation of the ISPMs, countries protect plant resources from fruit fly pests while, at the same time, they ensure the application of justified measures that facilitate safe trade.

Keywords: Host status, Fruit fly free areas, Systems approach, Tephritid fruit flies: fruits and vegetables, trade.

Session 10: Social, Economic and Policy Issues of Action Programs, November 18, 2022, 11:00 - 13:00

Medfly in a warming world: using models to understand shifting range dynamics

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Background: Climate is the principal factor defining the potential distribution of poikilotherms, and hence climatic changes are expected to influence the ranges of a range of pests, including insects, vector-borne diseases, and weeds. As these organisms spread beyond their native ranges, they may threaten natural and productive ecosystems, often triggering substantial expensive management responses.
Methods: We explore the apparent range expansion of Medfly, *Ceratitis capitata,* into areas that were hitherto too cold to support persistent populations, with a focus on Europe and California. We reviewed and refined a published CLIMEX model for *C. capitata*. We used the refined model to assess the climatic suitability of a range of recent occurrence locations spanning the previously modelled range limits in Europe. To assess the meaning of the new European distribution records for *C. capitata* we used the CRU TS4 climate time series dataset to explore the temporal patterns of climate suitability from 1970 to 2019.
Results: At selected indicator sites in Europe, we found statistically significant trends in increasing climate suitability, as well as a substantial northward expansion in the modelled potential range for persistent populations. Recent geographical records in Italy and France appear to represent a mixture of established and ephemeral populations, which is consistent with reports of the seasonal range dynamics of *C. capitata* in Europe. Over the same period in California, we also found a statistically significant trend of northward and altitudinal expansion of areas suitable for *C. capitata* persistence, and a general trend toward improved conditions for population growth.
Conclusion: These statistically significant trends in suitability for *C. capitata* are attributable to a warming climate. These findings have implications for both pest risk analyses and pest management of invasive fruit flies and other mobile pests. The modelling revealed the need to use a climatology that is temporally concordant with species distribution data.

Keywords: CLIMEX, Ceratitis capitata, medfly, niche modelling, pest risk analysis, distribution, climate change, seasonal phenology, invasion

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National fruit flies detection system (NFFDS) – Chile
(55 years of one successful plan)

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Background: The first detection of a Mediterranean fruit fly (*Ceratitis capitata* Wied) in Chile was in 1963 at the north of the country. The NFFDS was created in 1980 as: *Project for* Eradication *and Exclusion of Fruit Flies in Chile*, which was used as a guiding and unifying plan of different actions to fight against fruit flies that from time to time were intersected throughout the country. The project was approved as a state politics and its implementation was devolved upon SAG. Chilean fruit growing is the No. 1 item in agricultural and forestry exports with an amount of US$ 4,000 million returns. The cost for the Fruit Flies Program in Chile, its about US$ 7,8 million dollars per year.

Methods: The NFFDS protects 300,000 hectares of fruit production and its base is the permanent surveillance of fruit flies from Genus: *Ceratitis, Anastrepha and Bactrocera* through a network of 15,500 traps. In 1992 is built in the city of Arica the Sterile Insect Production Centre with a maximum production capacity of 50 million irradiated males; these insects are preventively released in the same city; and have also been used in case of Medfly outbreaks in the central region of Chile. The NFFDS success is sustained in 5 essencial columns:

1) Professional and Technical human team of excellence and committed with the Fruit Fly Program
2) Strict quarantine control at each point of entry into the country,
3) Permanent surveillance system in the country,
4) To have contingency's plans to respond quickly and effectively to any detection of exotic fruit flies in the country, and
5) Signature of agreements with neighbours countries on matters related to fruit flies.

Results and Conclusions: Incursion verification for Medfly in the country and of a very occasional detection *of Bactrocera dorsalis* in Easter Island (last detection 2011) and also detection *Anastrepha fraterculus* in the city of Iquique (located 1.700 kms north from the heart fruit production area). Thanks to the NFFDS and its 5 essencial columns tending to the early detection and eradication in the shortest possible time, maintains Chile as a free country for fruit flies of economic importance, for more than 55 years.

Keywords: NFFDS, country without fruit flies

Session 10: Social, Economic and Policy Issues of Action Programs, November 18, 2022, 11:00 - 13:00

Protecting the Australian way of life: 40 years of monitoring and eradicating exotic fruit fly in northern Queensland

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The Torres Strait in northern Queensland forms a pathway for exotic pests, diseases, and weeds found in neighbouring Papua New Guinea (PNG). In 1980 *Zeugodacus cucurbitae* was found in southern PNG and by 1983 a monitoring network of fruit fly traps was established in the Torres Strait (TS) and the Northern Peninsula Area (NPA) of Queensland. The Northern Australia Quarantine Strategy (NAQS) detected *Bactrocera dorsalis* in the Western Province of PNG in 1992, prompting an increase in the coverage of the trap network in the TS. By 1993 *B. dorsalis* was detected in the TS and an eradication program commenced.

Successful eradication of seasonal incursions of the exotic pest fruit flies *B. dorsalis*, *B. trivialis*, and *Z. cucurbitae* has been ongoing since 1993 and continues under the National Exotic Fruit Fly in Torres Strait Eradication Program. This program is a response under the Emergency Plant Pest Response Deed (EPPRD) and is delivered by Biosecurity Queensland (Queensland Department of Agriculture and Fisheries) with support from NAQS (Department of Agriculture, Fisheries and Forestry). A combination of Male Annihilation Technique (MAT) and bait spraying is used when target detections in monitoring traps exceed response thresholds as detailed in a formal Response Plan. If the eradication program did not exist an incursion of exotic fruit fly into mainland Australia would likely occur within 12 – 18 months.

Detections of exotic pest fruit flies are strongly seasonal, with the incursion season beginning in November and ending in May coinciding with the monsoon season. The bulk of detections occur in communities on Torres Strait islands adjacent to the Western Province of PNG. A collaborative Treaty Village Fruit Fly Trapping Program commenced in the Western Province in June 2022 with the aim of determining the origin of Torres Strait detections of *B. dorsalis* and investigating opportunities for fruit fly management to improve food security in the Treaty Villages.

Key words: Bactrocera dorsalis, Bactrocera trivialis, Zeugodacus cucurbitae, monitoring, eradication

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Two decades of fighting the invasive Oriental fruit fly *Bactrocera dorsalis* in Africa: A synopsis

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Background: Horticulture plays a pivotal role in providing income, food and nutritional security to millions of farmers in Africa. The cultivation of fruits and vegetables is lucrative but is constrained by various factors including fruit flies such as the invasive and devastating Oriental fruit fly *Bactrocera dorsalis*. The pest is widespread in Africa and since 2003 when it was reported for the first time in Kenya, various entities have been fighting the pest in various independent and sometimes collaborative approaches resulting in limited successes at the local level. A great deal of science has been generated, but it is debatable as to how much it has translated to adoption, and benefit for the farmer in monetary terms. The objective of this study was to explore the gains of research conducted on various aspects of *B. dorsalis* and interrogate them in light of successfully fighting the invasive pest.

Methods: We explored literature on various research outputs on *Bactrocera dorsalis* particularly peer-reviewed articles, and official reports. We have also collated information from numerous projects administered by various institutions and consortia within Africa. These have been collated and discussed based on facts, and opinions.

Results: The research has been impressive, largely funded by bodies outside Africa. African governments have done their part and collaborations between institutions in different countries either private or public have greatly contributed to this research. Technologies and products aimed at managing devastating pests have been developed and commercialised. There is a huge gap in technology development, transfer, and uptake. These are areas which require harmonization from the farmers' viewpoint in a bottom-up approach to optimize integrated pest management of *B. dorsalis*. National Institutions have not operationalised project outputs once funding ceases. Where trade is concerned, the establishment of pest-free areas, areas of low pest prevalence and postharvest treatments have been lagging. We discuss these findings in the context of seeking the way forward which must be anchored in the ideal future as seen from a farmer's perspective. We also discuss policy, and commitment by African governments and relevant institutions.

Conclusion: The greatest question will always remain What next? Can the gains accumulated in the past two decades be consolidated and what other mechanisms are required to facilitate technology and product transfer to accelerate uptake?

Keywords: Horticulture, Technology transfer, *Bactrocera dorsalis*, Trade, IPM, Fruit Fly

Poster: Biology, Ecology, Physiology and Behavior

Comparison of agroecological and conventional on management of fruit flies (Diptera: Tephritidae) infesting cucurbit crops in Morogoro, Tanzania.

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Background: Tanzania has experienced large crop losses due to true fruit flies (Diptera:Tephritidae) with increased incidence since 2004. Regardless the ecology of fruit flies has been extensively studied, there is limited information about how different management practices might affect their abundance and diversity. This study aims at comparatively describing patterns of infestation by cucurbit pests.

Methods: We compared the efficacy of selected agroecological and conventional practices on fruit fly management in cucurbit crops (*Cucumis sativus, Cucumis maxima and Citrallus lanatus)* under field condition across plateau (350-630 masl) and Mountainous (700-1400 masl) zones in Morogoro, Tanzania. A factorial experiment (2 × 3× 3) arranged in a Randomised Complete Block Design (RCBD) with five replications was set up. The treatments consisted of agroecological crop protection, conventional crop protection and control.-Agroecological practice included organic mulching, organic fertilizers, maize as trap crop, application of success bait (GF120) and augmentaria while conventional practices included industrial fertilizers, synthetic fungicides and insecticides. The experiment was conducted during the cropping season in 2020 (October-January) and 2021 (February- July). Data collection consisted of field observation on 45 plants randomly selected in 2025 m². Information was collected on number of fruits sets, number of fruits damaged during development, maturity, harvest and weight of marketable and non-marketable fruits. The data were transformed into percentage and subjected to a three-way analysis of variance. Significance of the main effect was tested with the post-hoc Tukey's-HSD test at a threshold of ($p<0.05$) using GenStat Discovery.

Results: This study showed the presence of *Zeugodacus. cucurbitae, Dacus vertebratus, D. ciliatus, D. frontalis, D. bivittatus and D. punctatifrons*. Further results revealed a significant difference in fruit damage percentage among the different crops and among the different management practices. The highest fruit damage percentage was observed in cucurbit crops that received no control practices (83.28%) followed by agroecological practices (56.02%) and conventional practices (41.98%). The most susceptible cucurbit crops were *C. lanatus* with 66.37% fruit damage while *C. maxima* (56.83%) and *C. sativus* (58.08%) were not significantly different C. maxima. Fruit damage was higher in plots at plateau zone compared to mountainous zone.

Conclusion: Agroecology showed a promising result compared to control plot while conventional practices had significant reduction in fruit damage as compared to agroecology management. Conventional, However, may result in many adverse conditions, particularly the loss of biodiversity of different life forms in cucurbit crop productions areas. Damage percentage may have an impact on the specific crop grown in particular areas.

Key words: Fruit flies pest, Cucurbitaceae, Agroecological zones, crop protection

Poster: Biology, Ecology, Physiology and Behavior

A first glance at stage-based life tables for the Lesser Queensland fruit fly, *Bactrocera neohumeralis* (Hardy) (Diptera: Tephritidae)

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Background: Life tables are a powerful tool to understand populations and their potential to increase or decrease over time but only a few examples are found for fruit flies. Experiments were performed to determine life table parameters for *Bactrocera neohumeralis* in two host fruit and a standard laboratory diet at a constant temperature (26 °C).
Methods: Eggs were collected from a laboratory colony, counted and infested into carrot medium, red papayas and Meyer lemons. Counts of egg hatch, pupation and adult emergence were performed at regular intervals and the difference in the median time from egg lay to each event occurrence was used to calculate days spent in each life stage. Adults which emerged from the same replicate were recorded, sexed and placed into cages for adult observation. Adult cages were checked regularly for dead individuals and offered a device for egg laying throughout female survival. Mean time for egg hatch, pupation and emergence of males and females for each trial were calculated and compared among substrates using linear mixed models. Random models identifying replicate sets of trials were fitted in the analyses where appropriate. Analysis was conducted in Genstat and all significance testing was performed at the 0.05 level. Life table calculations were performed in R following the age-stage two-sex life table theory.
Results: Mean egg hatch time was significantly greater for carrot medium (2.19 days) when compared to the two fruit types by approximately 3.4 hours (0.14 day). Mean pupation time differed among all substrates, taking significantly longer in lemons (12 days) when compared to carrot medium (8.6 days) and papaya (9.6 days). Mean adult emergence differed among all substrates, being faster in carrot medium (19.7 days), slower in lemons (23.8 days), intermediate in papaya (20.7 days). Proportional rates of survival from infestation differed for pupation and adult emergence, being lower on lemon. Adult reproductive period lasted from 91 to 126 days. Life tables presented rates of increase ranging from 0.07 to 0.09, mean generation time varied according to feeding substrate.
Conclusion: Life stage duration and mean generation time parameters differed according to immature stage food source. Knowing these parameters allow better understanding of *B. neohumeralis* demography and quantify how much a host may influence the fly's population increase and how fast that increase occurs. These life tables may be a tool to aid better timing of control measures and predict impact of the fly over time.

Keywords: demography, survival, fecundity, phenology, host

Poster: Biology, Ecology, Physiology and Behavior

A novel ABC gene involved in the interaction between unripe citrus fruits and *Bactrocera minax* larvae

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Background: The adaptation of phytophagous insects to host defense is an important aspect of plant–insect interactions. The Chinese citrus fly *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) is an oligophagous pest whose host range is exclusively restricted to unripe citrus fruits with abundant secondary metabolites. However, the mechanisms underlying the adaptation of tephritid fruit fly specialists, *B. minax*, to unripe host fruits remain elusive.

Methods: Plant metabolomes after infestation and insect transcriptomes were conducted for the first time to explore the interaction between unripe citrus fruits and *B. minax* larvae. RNAi and RT-qPCR were applied to reveal the function of the critical detoxification genes in *B. minax*.

Results: Eighteen citrus secondary metabolites, mainly flavones, alkaloids and phenylpropanoids, were identified in the unripe citrus fruits via metabolome, which accumulated during larval feeding. Three detoxification genes (1 P450 gene, 2 ABCs genes) were highly expressed in *B. minax* larvae collected from unripe citrus fruits compared with the ones fed on artificial diets and ripe citrus fruits. Based on omics data, a novel ABC gene was screened through plant allelopathy tests, and the gene was significantly upregulated in *B. minax* larvae treated with defensive secondary metabolites (N-Methylcytisine, tryptamine, coixol, limonin, nomilin and quercetin), respectively; additionally, the mortality rate of the larvae reached 51% after silencing the ABC gene by RNAi technique.

Conclusion: Overall, we clarified the variations of metabolites in unripe citrus fruits after larval infection. Importantly, a novel ABC gene was identified which plays a key role in host adaptation of *B. minax* larvae. These results shed light on the mechanisms underlying the biological interactions between tephritid fruit fly specialists and host fruits.

Keywords: Citrus fruit; Tephritid; Interaction; Plant secondary metabolite; Detoxification genes

Poster: Biology, Ecology, Physiology and Behavior

Age dependent exposure of *Zeugodacus cucurbitae* to semiochemical and assessing novel method of application to enhance mating competitiveness

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Background: *Zeugodacus cucurbitae* is a major pest of economic importance in Mauritius affecting over 30 % of the local cucurbits production. SIT is the environment-friendly technology selected to suppress this pest, and entails mass production and sterilizing of fruit flies using a radiation source. These procedures negatively impact on flies' performance. Exposures to semiochemicals have been effective in reducing the negative effects and improving sexual performance of flies. However, the age of exposure at which the flies respond mostly is a crucial parameter to be investigated. The mode of delivery of the semiochemicals should also be practical and compatible with the currently used systems for holding the mass-reared males in fly emergence towers and release facilities before fruit fly release.

Methodology: Lab reared irradiated flies were exposed to cuelure, provided on a filter paper, from day 2 to day 12 post emergence, and mating test was conducted at maturity for the first trial. In the second part of the trial, *Zeugodacus cucurbitae* flies were provided with agar adult diet prepared with cuelure (0.5 %, 0.6 %, 0.7 % v/v), and percentage mating was evaluated to assess the effect of the semiochemical.

Results: In part 1 it was observed that copulation was more successful for Day 7 exposures to Day 12 exposures. Response of males to cuelure increased with age and corresponded with sexual maturity of the flies. The highest mating record achieved was for Day 12 exposure, which corresponded with the peak in physical maturity of the flies. For Day 2 to Day 6 treatments, the number of mating pairs collected for both treated and non-treated flies was not significantly different, explained by previous findings that the effect of the cuelure is short-lived. The results of the second trial gave satisfactory results in terms of the mating competitiveness observed for the treated flies for all the different concentrations. Higher % mating were observed for treated flies. Moreover an earlier and systematic mating schedule for the treated flies also confirmed the beneficial effect of the cuelure provided in the adult feed.

Conclusion: Exposure to semiochemicals is recommended on day 7 following emergence if release is planned on Day 8 to maximise on the positive lure effect.

This novel mode of semiochemical supply is a potential avenue to be exploited in mass rearing facilities. Agar can be easily provided in paper bags where ground releases are implemented, and can be easily adapted for provision in emerging towers.

Keywords: *Zeugodacus cucurbitae, cuelure, age, mating competitiveness, agar diet.*

Poster: Biology, Ecology, Physiology and Behavior

Analysis of the interference between the small fruit fly *Drosophila suzukii* (Matsumura) and the fruit fly *Drosophila melanogaster* (Meigen) and its potential applications in biological control"

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Background: *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is an agricultural pest of international importance: the female is able to laid eggs in unripe small fruit of a wide range of species and endophagous larvae are inducing a severe damage with an important impact on the quality of the fruits and consequently on the yield. It is an alien species that from its native range in Asia rapidly moved and then became an invasive pest in part of North America, Europe and South America. Chemical insecticides still remain the primary method of control this species, but several alternative approaches are under investigation. Among them, biological control is of particular interest for sustainable management of this pest.
Methods: We investigated the potential interference in terms of interspecific competition between *D. suzukii* and *D. melanogaster*. First, we analyzed the population dynamics of the two species in monospecific and mixed lines in order to verify whether the interaction between the two species have a negative effect on the population growth of *D. suzukii*. Second, we assessed the relative role of larval and adult competition in affecting the population dynamics of *D. suzukii* by analyzing the fertility, development time and adult interference of the two species.
Results: By analyzing the population dynamics of *D. melanogaster* and *D. suzukii* we found that in monospecific lines, both species showed a logistic growth, although *D. suzukii* populations reach a significantly smaller size than those of *D. melanogaster*. In mixed lines, *D. melanogaster* showed a logistic growth reaching higher densities than monospecific lines, while *D. suzukii* population decreased and finally disappeared. By analyzing the possible factors underlying the observed population dynamics, we found that the female fertility did not differ between the two species. On the contrary, *D. melanogaster* showed lower development time than *D. suzukii*. Furthermore, by analyzing adult courtship behavior in single and mixed species pairs, we found that *D. melanogaster* males negatively affected the courtship between *D. suzukii* males and females.
Conclusion: Interspecific competition occurred between *D. suzukii* and *D. melanogaster* under laboratory condition, with *D. melanogaster* overcoming *D. suzukii.* Both larval and adult stages would affect the superiority of D. *melanogaster*. Although further studies under semi-field conditions are needed, these results support the possible use of competition with *D. melanogaster* as a possible control strategy of *D. suzukii* in confined environments, such as greenhouses.

Key Words: Spotted wing Drosophila, Competition, Biological control

Poster: Biology, Ecology, Physiology and Behavior

Artificial selection of body size: Effects on male competitiveness and life-history traits of *Anastrepha ludens*

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Background: Artificial selection of phenotypic traits that indicate better fitness, such as body size, represents a promising strategy to improve the quality of insects reared for application of the Sterile Insect Technique. However, the implementation of artificial selection demands the analysis of its effect on the fitness components in the offspring since there may be different trade-offs or phenotypic and genetic correlations between features that can cause indirect selection. For this reason, it is essential to evaluate the possible effects of selection on some life-history traits.

Methods: we performed a selection based on the pupae size in laboratory and wild populations, evaluating the effect on the male mating competitiveness and the life-history traits of their offspring over four generations.

Results: Our results showed that male size is not a determining trait in mating success in laboratory and wild populations. We also evidenced the adverse effects of colonization since, after one generation of rearing in laboratory conditions, wild males showed loss of sexual competitiveness compared to wild flies from the field. Here we also showed the bottleneck effects in some life-history traits such as life expectancy of selected males and females and fecundity in females, mainly in wild populations. Finally, we found that selection based on pupal size allowed to improve the body size of the selected populations after four generations of directional selection.

Conclusion: The domestication affected the performance of the wild population after one generation of laboratory rearing. We discuss the implications for SIT applications.

Keywords: experimental evolution, demographic parameters, sterile insect technique, phenotypic traits.

Poster: Biology, Ecology, Physiology and Behavior

Bactrocera tryoni from a constant temperature standpoint: how many generations can Qfly have in one year?

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Background: Meeting day-degree requirements is an essential intrinsic factor for insect development. Despite *Bactrocera tryoni* being the most economically important fruit fly in Australia, a comprehensive day-degree data set has never been generated for this species.
Methods: We reared *B. tryoni* on carrot media under six constant temperatures (14°C, 18°C, 22°C, 26°C, 30°C and 33°C) and 70% ± 10% RH. For each life stage, development time was recorded, and rate of development calculated. We fitted the generated rate-temperature dataset using three different models: Campbell_74 (33°C datapoints removed for linearity), Taylor_81 and Briere_1, using the R package devRate. Lower developmental thresholds (T_0) and optimum development temperatures (T_{opt}) were estimated and compared, while day-degree requirements were calculated.
Results: Using the linear model Campbell_74, T_0 varied from 10.4°C to 11.3°C depending on the life stage, and T_0 for overall development (egg to adult) was 11.1°C. In comparison, higher T_0 variation was suggested by the Briere1_95 model, ranging from 8.5°C (pupae to adult) to 15.8°C (adult reproductive maturation), and 8.7°C for overall development. The optimum calculated developmental temperatures (T_{opt}) were stage dependent, varying from 26.8°C (reproductive maturation) to as high as 35.1°C (pupae to adults), overall reaching maximum at 34.1°C in the Taylor_81 model. T_{opt} values were slightly higher in the Briere1_95 model than in the Taylor_81 model. The high calculated T_{opt}, particularly for the later life stages, were possibly due to the lack of datapoints at the high range of the rate-temperature curve rather than reflecting a true optimum temperature for development. Estimated using stage-specific T_0 values calculated from the Campbell_74 model, *B. tryoni* requires 284 ± 4 day-degrees to complete the egg to adult development, and a full egg-to-egg life cycle was 422 ± 35 day-degrees. At 26.8°C constant (optimum temperature for reproductive maturation calculated using Taylor_81 model) and with T_0 = 11.1°C, *B. tryoni* requires approximately 26.9 ± 2.6 days to complete a full life cycle.
Conclusion: At a constant 26.8°C, and assuming no other endogenous or exogenous factors influence its phenology and demography, *B. tryoni* can produce 13.6 ± 1.3 generations per year. For the egg to adult development, the lower developmental threshold was 11.1°C using the Campbell_74 model. Optimum development temperatures and T_0 (when applicable) can be estimated by fitting models Briere1_95 and Taylor_81, but the absence of datapoints to the right of the optimum range are likely to have influenced the estimation of T*opt*.

Keywords: Constant temperatures**,** day-degree, *Bactrocera tryoni*, life stages, development rates

Poster: Biology, Ecology, Physiology and Behavior

Covert viral infections in *Ceratitis capitata:* detection, distribution, and biological costs

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Background: The Mediterranean fruit fly (medfly) *Ceratitis capitata* is an agricultural pest of a wide range of fruits. The most extended strategy for medfly control is the Sterile Insect Technique (SIT) which relies in the mass-rearing and release into the field of sterile males of the species. In this context, the presence of pathogens and specifically viral infections represents a risk for the viability of mass-rearing and the success of SIT application. With the advent of high-throughput sequencing, a vast variety of Insect Specific Viruses have been identified in insects, including medfly. Majority of these viruses cause covert infections in which no evident symptomatology is displayed. However, previous studies with Ceratitis capitata nora virus (CcaNV) suggested a correlation between higher CcaNV abundance and shorter larval developmental time. With this work, we aimed to characterize the group of RNA viruses infecting medfly and analyse the influence of CcaNV in the normal development of the flies and the parasitism behaviour of their natural parasitoid *Aganaspis daci*.

Methods: High throughput sequencing (RNA sequencing, Illumina platform) and bioinformatic analysis were performed for viral discovery and characterization. Later, viral presence and relative abundance was assessed in mass-reared, laboratory-reared, and field-derived medfly strains by quantitative PCR. For the host-virus interaction studies, we orally infected CcaNV-free flies with CcaNV obtained from a naturally infected fly strain and we monitored pupal weight, adult emergence, flight ability and longevity under stress. Moreover, CcaNV-infected and Control flies were exposed to their natural parasitoid *A.daci*, to investigate the possible influence of CcaNV in the fecundity and fertility of the parasitoid.

Results: We expanded the RNA virome of medfly to a total of 13 viruses, including the first doble strand RNA viruses described for the species. The analysis of the viral abundance in different medfly strains demonstrated a great variability between strains except for two viruses, which were ubiquitously identified and therefore defined as core RNA virome of medfly. Regarding CcaNV, our results confirmed the presence of direct and indirect biological costs associated to CcaNV infection.

Conclusion: In summary, our work described the presence and distribution of 13 RNA covert viruses in *C. capitata* and highlights its influence over the application of biocontrol strategies, which could be affected by the biological costs associated to viral presence.

Keywords: Mediterranean fruit fly, covert infections, virus-host interaction, Sterile insect technique, natural parasitoids, pest control

Poster: Biology, Ecology, Physiology and Behavior

Cultivable gut bacterial diversity in fruit fly species of genus *Bactrocera* and *Zeugodacus* (Diptera: Tephritidae) from Bihar, India

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Background: Fruit flies of genus *Bactrocera* and *Zeugodacus* are economically important pests of fruit and vegetable crops in the world. Fruit flies are one of the major constrains in the export of fresh fruits and vegetables from India to other countries. Unavailability of suitable biocontrol agents and low toxicity pesticides for the management of fruit flies, demands for the application Sterile Insect Techniques (SIT) is growing day by day in India. However, irradiation process during sterilization makes sterile flies weak to compete with the wild flies. It has already been reported that some species of gut bacteria are very helpful to the sterile flies to make a strong competitor to the wild flies. Therefore, the gut bacterial diversity was explored from member of genus *Bactrocera* and *Zeugodacus* collected from different location of Bihar, India.

Methods: Adults of *Bactrocera dorsalis, Bactrocera zonata, Zeugodacus cucurbitae* and *Zeugodacus tau* were collected from different geographical locations of Bihar, India. Bacteria were isolated by standard protocol by surface sterilizing with sodium hypochlorite, ethanol treatment and sterile distilled water washing before dissection of flies to remove gut. Gut content of flies were serially diluted to get an optimum number of bacterial cell for pure culture preparation. Bacteria were grown on peptone yeast extract agar and Luria agar media for single colony isolation of different bacterial cells present on the culture plate. Bacterial isolates were identified with the 16S rRNA gene sequencing and bioinformatics tools. The 16S rRNA sequences of different bacterial isolates were submitted to GenBank with accession no. ON851019- ON851046.

Results: Total 31 bacterial isolates from adult fruit flies of different locations were pure cultured and among them 28 isolates were sequenced for 16S rRNA gene. Bacteria identified from different species of fruit flies with 16S rRNA gene sequences were from genus *Bacillus* (14 isolates), *Staphylococcus* (9 isolates), *Lysinibacillus* (3 isolates) and *Burkholderia* (2 isolates). *Bacillus* and *Staphylococcus were* isolated from the gut of fruit fly *B. dorsalis, B. zonata,* and *Z. cucurbitae.* Whereas, *Burkholderia* was only isolated from the gut of *Z. tau. Lysinibacillus* was only isolated from the genus *Bactrocera* in the present study.

Conclusions: *Bacillus* and *Staphylococcus* were widely present in the gut of fly *B. dorsalis, B. zonat* and *Z. cucurbitae.* Whereas, *Lysinibacillus* and *Burkholderia* was only present in genus *Bactrocera* and *Zeugodacus, respectively.*

Keywords: Gut bacteria, *Bactrocera*, *Zeugodacus*, 16s RNA, India, *Z. tau*

Poster: Biology, Ecology, Physiology and Behavior

Daily activity patterns of adult *Ceratitis capitata* (Wied.) under food and water deprivation

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Background: Detailed studies on daily activity patterns of model organisms may generate a wealth of data to answer both applied and basic biological questions. Specialized equipment makes possible to accurately study the organism behavior, stress response, etc. Aim of the current study was to explore the effect of food or water deprivation on the locomotor activity of adult Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae).

Methods: A modified LAM25 (Locomotor Activity Monitor; Trikinetics, USA) was used to monitor the activity patterns of 7-14 days old adult medflies subjected to various food and water deprivation treatments. Experiments were conducted under constant laboratory conditions (25±1.5 oC, RH 30±5%, Light:Dark 14:10h). Before being individually assigned into the LAM25 glass monitoring tubes, groups of virgin adults were kept in cages with water and adult food provision. On the water deprivation treatment flies were provided with crystalline sugar inside the glass tubes and had no access to water. On food deprivation treatment adults were provided with a water-soaked cotton fitted inside a plastic stopper at one side of the tube, which was re-soaked twice daily and had no access to food.

Results*:* Water and food-deprived males had higher day locomotor activity levels compared to the control flies during the 16-30 h after being placed on the deprivation treatment. Food-deprived males had higher night activity after 30-40 h compared to the control flies, while water-deprived males showed similar levels of night activity to the control at the same period. After 40 h, 77% of the food-deprived males were alive and their day activity was lower to the control. At the same time, 87% of water-deprived males were alive with day activity levels similar to the control. Food deprived females showed higher day activity during the 16-30 h after the beginning of the treatment and higher night activity (during the following 30-40 h) compared to water-deprived and control flies. After 40 h, all deprived females were alive with day and night activity levels similar to the control. After 64 h, both water and food-deprived flies (males and females) had day and night activity levels similar to each other but lower than the control group.

Conclusions: Food and water deprivation have an impact to the day and night locomotion activity of both sexes. Probably in search of food, they devote less time to resting during the day, or courtship behaviors, like male calling. Interestingly, the night activity is also increased, showing the inability of stressed flies to either initiate or sustain night sleep.

Key Words: locomotor activity, sleep, stress, Tephritidae, Mediterranean fruit fly, medfly

Poster: Biology, Ecology, Physiology and Behavior

Description of the regurgitation and bubbling behaviors in *Anastrepha fraterculus* (Diptera: Tephritidae)

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Background: Several fly species regurgitate droplets from the crop, which can settle in the proboscis while the fly remains motionless (bubbling) or be deposited on the surface of a leaf or fruit (regurgitation). Several hypotheses about this behaviour have been postulated, ranging from reproductive and nutritional functions to thermoregulation and detoxification. Even though regurgitation and bubbling have been described in many Tephritidae fruit fly species, little is known for *A. fraterculus*, and the information is limited to the description of the sporadic occurrence of these phenomena. In this sense, the aim of our work was to obtain a first comparative and descriptive behavioural record, from a broad perspective, of regurgitation and bubbling in *A. fraterculus*.

Methods: Bubbling and regurgitation were studied individually, in both sexes and at three ages, covering different times of the sexual maturation process. Experiments were carried out under controlled laboratory conditions, recording the number of individuals that regurgitated and/or were seen bubbling, as well as the duration and sequence in which each behaviour occurred. The number of drops deposited as well as whether they were later collected or not were recorded.

Results: Our initial assays revealed that flies from all treatments bubbled and regurgitated after feeding in similar proportions. Regardless of the sex or age, it took on average less than 10 min to initiate behaviour. In all cases, the most frequent initial behaviour was regurgitation (87% for both males and females), and then both behaviours occurred alternately while flies continued to consume food. The drops were collected by more than 85% of the flies.

Conclusions: This study provides the first characterization of the regurgitation and bubbling behaviours of *A. fraterculus*. So far, we did not detect differences associated with sex or age in the frequency of these behaviours, although males seem to take less time to initiate regurgitating, and deposit more droplets. The chemical characterization of the components of the extruded droplets, as well as behavioural tests under other contexts (such as mating or oviposition), will allow us to advance in the understanding of the roles that these behaviours could play in *A. fraterculus* ecology.

Keywords: South American Fruit Fly, crop, droplet extrusion, regurgitation

Poster: Biology, Ecology, Physiology and Behavior

Dirioxa pornia controlled temperature life stage to increase SA market access confidence

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Background: Although not a pest of export quality fruit, infesting over-ripe and damaged product, *Dirioxa pornia* (Island Fly) is regarded as a potential threat to Australian horticulture market accessibility. To inexperienced Biosecurity personnel the larvae could be misidentified as one of the economically important Tephritid fruit flies (e.g. Bactrocera spp. or *Ceratitis capitata*). The larvae have been found in visually intact fruit and look similar to economically important Tephritid fruit fly larvae, creating undue concern. Accurate life stage data tables of this fruit fly are needed to circumvent these issues and ensure market confidence. Effective control measures, facilitated by life table data, may also be used to reduce large populations of *D. pornia* in damaged fruit in orchards and the workload placed on SARDI and Biosecurity diagnostic services in the event of fruit fly outbreaks in South Australia.

Methods: Three substrates, oranges, avocados and an artificial gel diet, were inoculated with *D. pornia* eggs. Life stage data was collected two hourly for eggs during peak times and daily for pupae and adults until the commencement of oviposition. Data on the timing of life stage milestones, egg, pupae, and adult fly, at 3 constant temperatures (22^0C, 26^0C and 30^0C) were collected for life table data.

Results: At 26^0C, 54%RH, the majority of eggs hatched within 24 to 48 hours, from all substrates. Pupation occurred 11 days after egg inoculation on diet and 10 days after inoculation in both fruit. Maturation of eggs to adult flies was more variable, emergence occurred 20, 25 & 28 days after inoculation for avocado, diet and oranges respectively. Adults reached reproductive maturation when reared on diet at 17 days after emergence, from orange at 10 days and avocado at 11 days. Note: data yet to be analysed for other temperatures will be presented at the 11th ISFFEI Symposium.

Conclusion: Life history tables and development times on *D. pornia* reared on damaged orchard host fruit (avocado and orange) and artificial diet can give us a deeper understanding of the demographic and phenological occurrences of this species in South Australia orchards. This contributes to a deeper and more holistic understanding of Tephritid species and can facilitate a targeted response to manage fruit flies in orchards and prevent accidental occurrences in export fruit. These strategies can increase the confidence overseas and domestic trading partners such as Japan, New Zealand and China have in South Australian produce.

Keywords: market access, fruit fly, life history tables, *Dirioxa pornia*, egg, pupae, adult flies

Poster: Biology, Ecology, Physiology and Behavior

Dispersal behaviour of *Bactrocera dorsalis* in Réunion Island

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Background: Since its invasion in 2017 in La Réunion, the fruit fly *Bactrocera dorsalis* has caused considerable damage to crops of economic importance, such as mango. The sterile insect technique is a sustainable biological tool for managing fruit fly species (Tephritidae), and this technique is considered against *B. dorsalis* in La Reunion. Knowing the dispersal of released insects and estimating the size of field populations are keys to the success of area-wide management programmes based on the SIT, as they determine the release strategy of sterile males. Intending to set up the SIT in La Réunion to control *B. dorsalis*, we studied the dispersal of this species.
Methods: We used the mark-release-recapture experiment to study the dispersal behaviour of *B. dorsalis* in a mango production area of approximatively 50 Ha. Flies from a population from la Réunion were reared and sterilised by the IAEA, Vienne, and marked with fluorescent powder at the pupal stage. The sterile marked flies where kept in the laboratory of la Réunion until emergence and were released on day 0 in 2 distant sites of the studied area (1500m apart). 30 traps with methyl Eugenol and protein bait with insecticide was installed according to a grid, respectively every 200 or 400m, to recapture sterile males. Flies were allowed to disperse for 24h days 1, 3 and 5, and traps were open to catch flies for 24h days 2, 4 and 6. We identified marked sterile flies by observations to a numerical microscope with UV light. These mark-released-recapture experiments were conducted several times in different seasons.
Results: We determine the proportion of marked flies in each trap and release events according to the distance and the capture timing. These results allow us to estimate the size of the field population, survival and mathematical indexes to describe the movement of insects in the field.
Conclusion: Those results have important practical implications for area-wide management strategies for *B. dorsalis*. Our estimated dispersal parameter could be integrated into the model to determine the number and frequency of sterile male releases.
Keywords: SIT, Réunion island, mark release recapture, dispersal, Bactrocera dorsalis

Poster: Biology, Ecology, Physiology and Behavior

Distribution and host plants of *Bactrocera cucurbita, Bactrocera dorsalis and Bactrocera invadens* in 5 Agro ecological zones in Cameroon and it impacts on IPM in the field

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Background: About two decades ago, crops have been affected by fruit flies (Diptera Tephritidae) and especially the genus *Bactrocera* in Cameroon. Three species are very rampant on mango (*B. dorsalis* and *B. invadens*) and garden crops (*B. cucurbitae*).
To date, the technologies mostly available to farmers in Cameroon for phytosanitary control are mostly the use of synthetic chemicals. The lack of knowledge on distribution and behavior of pests, leads to: misuse of chemicals, pest resistance and resurgence and damage to the health of humans, animals and the environment.
It is important to collect data on these three pests in order to understand their behavior and contribute to IPM strategies.
Methods: This work was completed in each agro ecological zones in Cameroun. A total of 30 traps were set up in the field for weekly data collection from 29th November 2021 to 19 May 2022, at the beginning of the flowering season of mangoes. We used Methyl Eugenol for *B. dorsalis and B. invadens*, Cuelure for *B. cucurbitae.* The active ingredient of insecticide stick was Dichlorvos. We used GPS positions and data collected for mapping and improve the IPM strategies.
Results: The insect collection made it possible to have a total of 146 106 insects.
B. cucurbitae is present in three agro ecological zones. The number of insects in Western Highlands area is the highest, respectively 51 and 31 times more than in Bimodal rainforest and Sudano sahelian zones. This is due to the fact that, many farmers of market gardening crops mostly cultivate during the same period**.**
B. invadens and B. dorsalis are the pests infesting mangoes in Cameroon from flowering to harvest periods and establish in every zones. The cumulative number of insects is the highest in monomodal rainforest zone because of the: early flowering from December, high temperature and highest annual rainfall. There is a correlation between the pest increase population, temperature, rainfall and diversity of host plants.
In general, it will be good to conduct a phytosanitary surveillance including any good agricultural practices from two weeks before flowering and start early mass trapping in order to reduce pest establishment level. The issue of cultivated or wild host plants should be considered.
Conclusions: The repeated trapping period conducted and densification of trapping network will give good understanding of pest behavior. It's very important to come out with economic threshold of pest population specific to each zone. Knowledge on natural enemies will help for IPM strategies.

Key Words: Fruit fly, good agricultural practices IPM strategies, Mass trapping

Poster: Biology, Ecology, Physiology and Behavior

Diversity and Abundance Pestiferous Fruit flies (Diptera: Tephritidae) Infesting Cucurbit Crops in Morogoro, Eastern-Central Tanzania

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Background: The production of horticultural crops in Tanzania is under threat due to fruit fly infestation on important vegetable crops such as cucurbit crops. Fruit flies (Diptera: Tephritidae) are globally recognized as major pests of horticultural crops. They inflict huge economic losses ranging between 30% and 100% on cucurbit crops.

Methods: We investigated the diversity and abundance of fruit flies infesting cultivated cucurbit crops across the two agroecological zones of the Morogoro region. Attractants (Cue Lure, Zingerone and Biolure) were deployed and weekly catches of fruit fly species were recorded. The diversity of fruit flies was evaluated using both alpha(α) and beta (β) diversity indices, and effects of agroecological zones, seasons and fruit fly species were determined using analysis of variance.

Results: A total of 11634 fruit fly individuals were collected, of which 77.3% were collected from the mountainous and 22.7% from the plateau zone. *Zeugodacus cucurbitae* was the most abundant species accounting for 80.2% of the total catches, while the remaining species constituted 19.8%. The species diversity varied significantly across the two agroecological zones ($P<0.05$). The mountainous zone was the most diverse and species-rich zone (Shannon = 1.32, Chao1= 9.4) compared to the plateau zone (Shannon =0.96, Chao 1= 4). The two agroecological zones were highly dissimilar in terms of species composition (Sorensen=0.52). The abundance of fruit fly species was significantly influenced by agroecological zones and seasons ($P<0.05$).

Conclusion: The study confirms that the diversity and abundance of fruit flies is higher in the mountainous zone than in the plateau zone, and *Z. cucurbitae* is the most prominent species in both zones. Therefore, management programs against cucurbit infesters should be more intensive and centred on *Z. cucurbitae* regardless of the agroecological zones.

Keywords: Diversity, Species richness, Seasonal abundance, Fruit flies, Cucurbit crops

Poster: Biology, Ecology, Physiology and Behavior

Effect of fruit volatiles from native host plants on the sexual performance of *Anastrepha fraterculus* males

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Background: The SIT requires good quality of sterile males to compete against wild fertile males. The South American fruit fly, *Anastrepha fraterculus*, is one of the main fruit fly pests in the region and together with *Ceratitis capitata* cause significant economic losses. *Anastrepha fraterculus* males increase their sexual perform after exposition to the volatiles of *Psidium guajava* (Myrtaceae), one of the most important native host for this species. Here we analysed the effects of fruit volatile exposure of other native host plants on male sexual performance.

Methods: Four native plant were evaluated: *Hexachlamys edulis*, *Psidium cattleianum*, *Acca sellowiana* (three species of Myrtaceae), and *Juglans australis* (Juglandaceae). Furthermore, *P. guajava* was used as positive control. After emergence of flies, groups of 30 males were placed in 3 L glass containers with food (sugar and brewer's yeast, 3:1) and water, and kept in chambers with controlled environmental conditions (25ºC, 12 h of light). During exposure, 10 small pieces (ca. 1 g each) of the evaluated fruit were placed in 20 mL containers that were covered with a mesh to prevent contact. This container was placed into the containers with males during four hours (12:00 pm– 4:00 pm) from day 8 to day 11 post emergence. Control males were not exposed and maintained in separated chambers. Females were maintained in similar conditions, without exposition. At day 12 post emergence, males were evaluated in two experiments: calling behaviour tests (in which the frequency of fanning and exposition of salivary glands was recorded), and sexual success test (each exposed male competed against other non-exposed male for the access to a female in small mating arenas -1L container-).

Results: In agreement with previous works, *P. guajava* enhanced calling behaviour and mating success of males. Although it was not as strong as *P. guajava*, *P. cattleianum* also enhanced these parameters. The other native fruits showed not trends to improve calling behaviour or mating success of males.

Conclusion: Only one of the four native host fruits tested here positively affected calling behaviour and mating success in *A. fraterculus* males. Interestingly, the two that induced this increased competitiveness belong to the genus *Psidium*. Volatile analysis of these fruits is planned to identify the compounds responsible of this phenomenon. These results can be used to support the SIT against *A. fraterculus.*

Keywords: Chemical ecology, Semiochemicals, Fruit Volatile, Sterile Insect Technique, Tephritidae**.**

Poster: Biology, Ecology, Physiology and Behavior

Effect of gut-symbiotic community on nutrient reserve of *Bactrocera dorsalis* and *Zeugodacus cucurbitae* (Diptera:Tephritidae) under diet and temperature stress

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Background: Insect microbiota, particularly, gut symbionts has recently gained especial attention in Tephritidae fruit flies. Symbiotic bacterial communities that colonize the digestive tract of tephritid fruit flies interact with nutrient intake to improve the flies' fitness and immunity through several life-history traits. It is often assumed that some of these symbionts play crucial roles in the nutritional physiology of their insect hosts. The interaction among gut bacterial community in two invasive fruit flies in la Réunion, *Bactrocera dorsalis* and *Zeugodacus cucurbitae* (Diptera:Tephritidae) was deciphered using different quality of diet under a range of temperature.

Methods: The role of gut symbiotic community on the nutritional status of *B. dorsalis* and *Z. cucurbitae* females that were fed on manipulated diet was tested. For this purpose, wild females (axenic) were reared on a diet with antibiotic (200 µg/ml streptomycin, 45 µg/ml kanamycin, and 15 µg /ml of gentamycin) and compared to wild females (non-axenic) reared on diet without antibiotic. These females were raised on three types of diets: two in which carbohydrate or protein contents were reduced and one control diet. This experiment was repeated under three temperatures (10, 25, and 30°C). Using biochemical techniques, the amounts of lipid, carbohydrate and protein in each individual were established quantitatively, and six replicates were run for each treatment group.

Results: Gut symbiont loss in axenic flies, adult food quality, heat stress affect significantly adult survival and fecundity of *B. dorsalis* and *Z. cucurbitae***.** Effect of antibiotic treatments on lipid, carbohydrate, and protein reserves in wild fruit fly females reared under different food quality and heat and cold stress are discussed.

Conclusion: These findings in combination with those of earlier studies provide base line information for future investigations in the possible functional role of the observed associations.

Keywords: Gut-symbionts, Antibiotics, Nutritional reserves, Diet diversity, Temperature

Poster: Biology, Ecology, Physiology and Behavior

Effect of limited protein consumption during the larval and adult stage on life-history traits of the Mexican fruit fly

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Background: *Anastrepha ludens* Loew (Diptera: Tephritidae) is an important mango and citrus pest. The sterile insect technique (SIT) is currently used to control this fly. The SIT involves the release of sterile insects to reduce wild populations through the induction of sterility. Prior to release, insects are mass-reared on artificial diets. The rearing of *A. ludens* involves the provision of protein both in the larval and adult stage, thus we studied trade-offs between larval and adult diets.
Methods: We compared various yeast concentrations in the larval diet and two formulations in the adult diet, both at small and semi-mass scale. We determined the quality of the insects at all developmental stages.
Results: Low concentrations of torula yeast (TY) (2.9 and 3.6%) produced larvae and pupae of low weight, fewer larvae and pupae, and more males than higher TY concentrations (6.1%), but this was only discernable at a small scale. Adult survival without water and food was significantly lower for non-irradiated males and females, and irradiated males when reared on 6.1% TY. For mating success, we found that non-irradiated males reared on 4.8% TY but fed only sugar (S) as adults had lower probability of mating than males fed yeast hydrolysate (YH). For irradiated males, larval diet had no effect on mating competitiveness, only males fed with S+YH in the adult diet had higher mating success than those fed with S only.
Conclusion: Reducing TY in the larval diet to 5.1%; however, for release colonies S+YH will need to be provided in the adult diet. Knowledge on larval and adult nutritional requirements will aid in our understanding of insect nutritional ecology and will also improve mass-rearing diets.

***Key Words*:** *Anastrepha ludens*, artificial diets, Diptera, mango and citrus pest, mass rearing, SIT, sterile insect technique, Tephritidae, trade-off, yeast hydrolysate.

Poster: Biology, Ecology, Physiology and Behavior

Environmental sensitivity of *Ceratitis capitata* (Diptera: Tephritidae) wing shape an example in Croatian populations

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Background: The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) is one of the most economically important fruit flies globally. Its development depends on the geographic area, climatic conditions, and availability of host plants. Given its cosmopolitan distribution, invasive nature and its abundance and ability to thrive in numerous environments, medfly is an ideal model species to investigate the effects of environmental factors on size and shape variation. Geometric morphometrics (GM) can provide valuable information on phenotypic variability and population structure. Using geometric morphometric tools, the size and shape of the wing can be analysed independently to untangle biological patterns related to heritability and environmental sensitivity.
Methods: Adult medfly populations were developed from peach that was infested in the field from two Croatian locations with different agroecological conditions and different pest management practices. First, Podstrana with conventional pesticide control and second Opuzen with sterile technology avoiding the use of pesticide. Wings of each fly were removed and laterally fixed according to the standard methods for subsequent morphometric analysis. On each medfly wing, 14 landmarks were placed at the nodes or ends of the veins. To distinguish between sexes and populations, a principal component analysis (PCA) was performed using the covariance matrix of the individuals' wing shape and differences corroborated by a Procrustes ANOVA. A multivariate regression was performed to analyse whether there was an effect of size on wing shape (Allometry).
Results: Results shown significant differences between medfly sexes and populations where shape variation was analysed independently by sex. The female medflies have more elongated and larger wings compared to the males. These wings are more aerodynamic and less prone to flight damage. This allows the females to fly more frequently and for longer periods of time, making them responsible for dispersal into new areas. Wing size and shape differ among populations with significant differences between agroecological systems and pest management practices.
Conclusion: Wing size and shape showed variations between different agroecological systems suggesting metapopulation structure of medfly which is currently favouring sex-specific plasticity. These results show that geometric morphometric procedures can be used to provide key insight into the biological adaptation of medfly in newly invaded habitats, a significant process that ensures invasion success.

Keywords: medfly, geometric morphometrics, peach, wing shape, invasion, variability

Poster: Biology, Ecology, Physiology and Behavior

Food deprivation of *Bactrocera oleae* (Rossi) adults and survival at various ages

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Background: The effect of age on survival of olive fruit fly (*Bactrocera oleae*, Rossi) adults under conditions of food deprivation was studied. Although there are published works on the survival of the olive fruit fly in relation to the type of food it consumes, the resistance (survival) of the insect to food deprivation conditions has not been studied.

Methods: The effect of the age of females and males of the olive fruit fly in food deprivation conditions was studied in the laboratory (25 °C). Adults were placed in individual cages from the first day of life with water and food (a mixture of hydrolyzed protein and sugar). To record the resistance (survival) to food deprivation, ten females (0 to 121 days) and ten males (0 to 88 days) of a certain age were each transferred to a new individual cage from which the food was absent. Their survival was recorded every 4 hours.

Results: It was found that young females aged 1 - 30 days are more resilient than older adults. In the age classes 31-60, 61-90 and 91-130 the resilience continues to decrease slightly with age, but the observed differences are not statistically significant. Similarly, young males aged 1 to 30 days are more resilient than older males. In the two older age classes (31-61 and 61-90 days) there is a mild downward trend with age which is however not statistically significant. The functions found to best express the decline in food deprivation resistance with age are polynomial for both genders. In all age groups females appear to be more resilient than males. However, a statistically significant difference is observed only in the age class 61 - 90 days.

Conclusion*:* The differences observed in survival are most likely related to the food stocks of adults at different ages. Young adults may carry food stocks from the larval stage, which are depleted at older ages. In the case of females, these differences are probably also strongly linked to their reproductive maturation. These results contribute to the understanding the biology and survival mechanisms of the olive fruit fly in adverse environmental conditions and aid in the quest of effective management approaches.

Key Words: olive fruit fly, food stress, aging, starvation, pest management

Poster: Biology, Ecology, Physiology and Behavior

Fruit flies (Diptera: Tephritidae) in Mango Plantations in Vientiane Province, Laos.

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Background: Increasing areas of Vientiane Province are being devoted to fruit tree plantations, including large numbers of mangoes. These mangoes are infested with numerous pests, most seriously tephritid fruit flies. Fruit flies are poorly understood in Laos with only about 30 species recorded and these from just a handful of localities. Research on phenology, economic impact and management is in its infancy in the country. This study records diversity and relative abundance of tephritids in mango plantations in Vientiane Province and is a first step towards developing locally tailored, management strategies for these widespread pests.

Methods: Trapping using Methyl eugenol (ME) lures was conducted over 10 weeks from early March to May 2019, which included the onset of the annual wet season. Traps were hung in the middle of mango trees at a density of 1 trap per hectare (total 10 traps). Data, including temperature and relative humidity, and samples were collected weekly and the lures replaced every month. Specimens were identified based on morphology.

Results: Four species of fruit flies were recorded: 55,108 *Bactrocera dorsalis* (Hendel), 28,235 *Bactrocera correcta* (Bezzi), 67 *Zeugodacus cucurbitae* (Coquillet) and 4 *Zeugodacus tau* (Walker), i.e. 66.06%, 33.85%, 0,08% and 0.01% of catch respectively.

Conclusion: *B. dorsalis* was most commonly trapped, followed by *B. correcta*, reflecting that both are pests of mangoes and that *dorsalis* has a greater preference for mangoes than *correcta*. These species should be targets for management, e.g. by timely bagging of fruit. The tiny catches of *Z. cucurbitae* and *Z. tau* reflect that ME is a poor attractant for these species and that neither species prefers mangoes as a host.

Keywords: *Bactrocera* spp., *Zeugodacus* spp., mango, Laos

Poster: Biology, Ecology, Physiology and Behavior

Fruit fly community structure on cucurbits crops grown under agroecological and conventional farming practices

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Cucurbits are important vegetable crops grown all over the tropical world for income generation, food and nutrient security. However, their production is threatened by several biotic and abiotic factors, including "true" fruit flies (Diptera, Tephritidae) that are mainly controlled by using pesticides. Due to the negative impacts of pesticides, agroecological practices have been adopted in crop production to reduce or eliminate the use of pesticides. This study evaluated how the diversity and abundance of fruit fly pest changes between agroecological and conventional farming.
Field experiments were carried out in Morogoro and Mvomero districts in Tanzania at the mountainous (approx. 500 m a.s.l.) and plateau zones (approx. 1000 m a.s.l.). Ten experimental plots (replicates) of 45m × 45m were established at each altitude. The plots were randomly assigned to conventional or agroecological farming and planted with three types of crops; cucumber, watermelon and squash. Laboratory experiment was set as a 2x3 factorial experiment in completely randomized design with 10 replications. Cucurbit-infesting fruit flies were monitored for 7 weeks in April, May and June 2022 after fruit development. Sampling was conducted biweekly, fruits were randomly collected, counted, weighed and placed in separate rearing cages for rearing experiment in the laboratory. Following 14 days of incubation emerged fruit flies were collected and unmerged fruit flies in the pupae stage were kept further in Petri dishes until adults emerged. Emerging adult fruit flies were collected, counted and morphologically identified to species level and preserved in vials with 100% alcohol.
Fruit fly species diversity was determined by using α and β diversity indices. Species richness was estimated through the Chao estimator. The effects of covariates were evaluated and compared between agroecological and conventional cucurbit production by a mixed model.

The study will comparatively describe the spatial and temporal dynamics of fruit fly assemblages between agroecology and conventional farming and will provide a basis for scrutinizing the cost-benefits of agroecological farming practices over conventional ones, which in turn will allow more sustainable control measures to be implemented/devised.
Keywords; Agroecology, Fruit fly, Cucurbits, Fruits, Vegetables.

Poster: Biology, Ecology, Physiology and Behavior

Gut symbiotic bacteria involved in nitrogen recycling in the tephritid fruit fly *Bactrocera dorsalis*

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Background: Nitrogen is considered the most limiting nutrient element for herbivorous insects. To alleviate nitrogen limitation, insects have evolved various symbiotically mediated strategies that enable them to colonize nitrogen-poor habitats or exploit nitrogen-poor diets. In frugivorous tephritid larvae developing in fruit pulp under nitrogen stress, it remains largely unknown how nitrogen is obtained and larval development is completed. In this study, we uncover the mechanism underlying the nitrogen exploitation in the larvae of *Bactrocera dorsalis*, a notorious tephritid insect pest, infesting more than 250 different fruits and vegetables.

Methods: We used metagenomics and metatranscriptomics sequencing technologies to analyze the diversity of bacterial communities and investigated the potential functions of bacteria across different life stages of *B. dorsalis* (pupae, larvae and adults). Combining with *in vitro* verification tests (including acetylene reduction assay, isotopic tracer technique, quantitative urease assays), we compared the completeness of the biological nitrogen fixation (BNF) and nitrogenous waste recycling (NWR) pathways in the *B. dorsalis* microbiota.

Results: Our results showed that NWR could be successfully driven by symbiotic bacteria, including Enterobacterales, Lactobacillales, Orbales, Pseudomonadales, Flavobacteriales and Bacteroidales. In this process, urea hydrolysis in the larval gut was mainly mediated by *Morganella morganii* and *Klebsiella oxytoca*. In addition, core bacteria mediated essential amino acid (arginine excluded) biosynthesis by ammonium assimilation and transamination.

Conclusions: Based on the fact that genes related to BNF or NWR are missing in *B. dorsalis*, we propose that (1) symbiotic bacteria contribute to nitrogen transformation in the larvae of *B. dorsalis* in fruit pulp; (2) the pattern of NWR is more likely to be applied by *B. dorsalis* due to its energy conservation and mild reaction conditions; and (3) *M. morganii*, *K. oxytoca* and other urease-positive strains play vital roles in hydrolysing nitrogenous waste and providing metabolizable nitrogen for *B. dorsalis*.

Keywords: *Bactrocera dorsalis*, nitrogenous waste recycling, biological nitrogen fixation, amino acid biosynthesis, urease activity

Poster: Biology, Ecology, Physiology and Behavior

Harmonic radar tracking of *Bactrocera* and *Zeugodacus* fruit flies

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Background: Tephritid fruit flies, such as the Queensland fruit fly (Qfly), *Bactrocera tryoni*, and the melon fly, *Zeugodacus cucurbitae*, are major horticultural pests worldwide and additionally pose invasion risks. Determining movement parameters for fruit flies are critical to implementing surveillance and control strategies, particularly in the development of agent-based models. While mark-release-recapture, flight mills, and visual observations have been used to study tephritid movement, each of these techniques have limitations. Tracking tagged flies offers an alternative method which has the potential to observe individual fly movements.
Methods: Harmonic radar (HR) tags were fabricated using superelastic nitinol wire which is light (tags weighed less than 1 mg), flexible, and does not tangle. Multiple tephritid species were tagged and subjected to flight tests. Subsequent experiments successfully tracked HR tagged Qflies and melon flies in a number of environments including large field cages, papaya fields, and open parkland.
Results: Flight tests with multiple tephritid species showed mixed results in terms of adverse effects for HR tag attachment. Qfly, melon fly, *B. dorsalis*, and *B. jarvisi* wild males were all capable of flight when tagged. Individual flight distances (meters per flight) for male melon fly differed between the field cage, papaya field, and open field experiments with longer mean step-distances observed in the open field. Both melon fly and Qfly males showed similar step frequency vs. step distance relationships. A majority of tagged male melon flies showed strong flight directional biases with these biases varying between flies, similar to what has been observed in the migratory butterfly *Pieris brassicae*. In field cages experiments, 83% of male melon flies showed directionally biased flights while similar biases were observed in roughly half the flies tracked in the papaya field. At least some of the direction bias can be explained by wind direction with a correlation observed between overall fly flight directions in the field cage, papaya field, and open field experiments. However, individual melon fly mean flight directions coincided with the observed wind direction for 9 of 25 flies in the cage experiment and half the flies in the papaya field experiment, suggesting wind is unlikely to be the only factor affecting flight direction.
Conclusion: Data on flight directions and step-distance determined in this study might assist in the development of more accurate agent-based modelling for pest tephritid species. Additionally, harmonic radar tags developed in this study allow tracking tephritid fruit flies in a variety of environments. We believe this will enable new areas of study on tephritid movement, phenology, and behaviour which will potentially improve detection and control methods.

Keywords: Modelling, field tracking, cage bioassay, step distance, flight direction

Poster: Biology, Ecology, Physiology and Behavior

Host preference and associated performance in *Anastrepha fraterculus* (Diptera: Tephritidae)

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Background: *Anastrepha fraterculus* (Diptera: Tephritidae) is major fruit pest in Argentina. Despite being a highly polyphagous species, not all plant species are equally infested in nature, which might point to a degree of preference among the different host available. Because immature stages are bounded to develop in the host selected by their mother, oviposition preference in females has been linked to the performance of the progeny (preference-performance hypothesis). Here, we studied the preference of *A. fraterculus* females and addressed the effect of developing in specific host fruit on the performance in *A. fraterculus*.
Methods: the preference-performance hypothesis was tested on 5 on a subset of representative host species: loquat, peach, plum, guava and feijoa. Preference was assessed in no-choice trials, exposing fruit fly females to the different fruits in lab cages and recording their behaviour. After females laid eggs in each one of the tested fruit, the samples were kept and developmental parameters, as well as adult parameters, were recorded.
In the preference test, we recorded the latency to visit the fruit, the latency to oviposit, and the permanence on the fruit. After fruit was infested, we evaluated the percentage of adult emergence, the egg-pupae developmental time, sexual maturation time in males and starvation resistance of emerged adults.
Results: The results showed that loquat and guava were the most rapidly visited fruits and where more ovipositions occur. In addition, oviposition occurred more frequently in these two host species. The permanence on the fruit and the oviposition activity of the females was higher in feijoa and guava. Regarding the variables related to performance, the percentage of emergence was higher in peach. The adults emerged from loquat were able to resist starvation for longer periods of time. Males emerged from plum reached sexual maturity more quickly.
Conclusion: We can conclude that or *A. fraterculus* the preference-performance hypothesis will depend on the fruit species evaluated.

Keywords: fraterculus, fruit, performance, preference.

Poster: Biology, Ecology, Physiology and Behavior

Host use preference of fruit flies (Diptera: Tephritidae) among selected cucurbitaceous vegetables in Morogoro, Eastern-Central Tanzania

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Background: Fruit flies (Diptera: Tephritidae) are among the serious pests of fruit and vegetables in Tanzania. They cause huge economic losses on horticultural crops such as cucurbit crops. Due to their polyphagous nature and potential to limit access to international markets, fruit flies have been considered the major threat to cucurbit production in Sub Sahara African countries.
Methods: We investigated the incidence and infestation rates of cucurbit infesters from three cucurbit crop species; cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) and squash (*Cucurbita maxima* L.) planted in ten cucurbit plots established across the two agroecological zones of the Morogoro region. A weekly sampling of five to twenty cucurbit fruits was done for five consecutive weeks in each cucurbit growing season.
Results: Results showed a significant difference in host use preferences among fruit fly species. Squash was the most preferred host by *Zeugodacus cucurbitae,* watermelon for *Dacus vertebratus* and cucumber for *Dacus ciliatus.* The incidence of *Z. cucurbitae* was significantly associated with the host crop ($P<0.05$). On the other hand incidence of *D. vertebratus* was significantly associated with host and altitude ($P<0.05$). Likewise, the incidence of *D. ciliatus* was significantly associated with host, altitude and season ($P< 0.05$).
Conclusion: All dominant fruit fly species had significantly higher infestation rates in all cucurbit crops. The type of cucurbit crops, altitude and season are the influential factors for the higher incidences and infestation rates among the fruit fly species. Therefore, management programs should focus on the suppression of the fruit fly population on the hosts regardless of seasons and altitude

Keywords: Host use, incidence, infestation, fruit flies and cucurbit crops.

Poster: Biology, Ecology, Physiology and Behavior

Identification and expression profiles of gustatory receptor genes in *Bactrocera minax* larvae (Diptera: Tephritidae): Role of *BminGR59f* in larval growth

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Background: Insects employ various types of gustatory receptors (GRs) to identify nutrient rich food and avoid toxic substances. The larval gustatory system is the critical checkpoint for food acceptance or rejection. As a specialist herbivore, *Bactrocera minax* larvae feed only on unripe citrus fruits which contains a large variety of bitter compounds. However, how the *B. minax* larvae use GRs to recognize and adapt to the secondary metabolites in unripe citrus fruits remains unknown.

Methods: In this study, we annotated BminGRs based on the genome and transcriptome data of *B. minax*. Moreover, RT-qPCR was performed to detect the expression level of *BminGRs*. Further, RNAi and cell calcium imaging were applied to explore the function of *BminGRs* and screen the corresponding ligands.

Result: Fifty-one BminGR genes were identified in *B. minax* larvae. Based on phylogenetic tree analysis, we found that most gustatory receptors in *B. minax* are closely related to *Drosophila melanogaster.* RT-qPCR results showed that most BminGRs highly expressed in the 1st and 2nd instar larvae and presented high expression in the mouthparts of the 2nd instar larvae. Meanwhile, silencing of *BminGR59f* by RNA interference (RNAi) significantly reduced the growth of 2nd instar *B. minax* larvae. Furthermore, hesperidin and naringin were screened as ligands of *BminGR59f* via RNAi and cell calcium imaging, and the combination of two flavones increased the body weight of larvae.

Conclusions: In summary, we identified a novel gustatory perception pattern in the larvae of *B. minax* for handling unripe citrus fruit secondary metabolites. These results provide better understanding on how specialist herbivores detect and adapt to host metabolites depending on larval GRs in adverse environments.

Key words: Gustatory receptors; Secondary metabolites; Specialist herbivores; Tephritid; Unripe host fruits

Poster: Biology, Ecology, Physiology and Behavior

Interaction between two species of fruit flies (Diptera: Tephritidae), *Bactrocera dorsalis* Hendel and *Ceratitis malgassa* Munro

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Background: horticultural crops are particularly threatened by insect pests, including several species of fruit flies belonging to the Family Tephritidae. In Madagascar, two polyphagous species of this family are considered to be the major pests of fruit crops, one of which is an invasive species, *Bactrocera dorsalis*, and the other is endemic, *Ceratitis malgassa*. These two species with very close host ranges can be found in sympatry in the same fruit. Since the arrival of *B. dorsalis* on the island (2010), high populations of this species were recorded. The main objective of this study was to understand the adult and larvae interactions between these two species in relation to resource access.

Materials and methods: Interaction between adult was quantified by placing a combinations of two mature females flies (*B. dorsalis * C. malgassa*, *B .dorsalis * B. dorsalis*, *C.malgassa * C.malgassa*) in a cage as well as exposing them to three natural host fruits (banana, guava, and strawberry guava). In the control cages, the behaviours of each species (on each different tested fruit) were observed separately. The flies behaviours were observed for 30 min for each experiment. Each experiment was replicated 20 times.

At larval stages different co-infestations of both species (with different larval ratio 50:50, 0:50, 50:0, 25:75, 75:25, respectively) in the same host fruit (several hosts were tested strawberry guava, banana and guava) were carried out. The experiments were performed in a laboratory maintained at 25°C ±1°C, 75 ± 5 % RH, and a photoperiod of 12:12 (L:D).

Results: Under competitive interaction, aggressive encounters in the form of lunging/ head-butting and chasing off other species from fruits was significantly higher for B. *dorsalis* compared with *C. malgassa*. Females of *B.dorsalis* significantly oviposited on fruit hosts with greater frequency than *C. malgassa*. In the mixed species group at larval (50:50), the proportion of emerged *B. dorsalis* adults (74.58±23.75 % in strawbery guava, 56.45±3.45 % in guava, 52.61±4.77 % in banana, respectively) were significately higher than *C. malgassa* (21.50±17.66 % in strawbery guava , 41.91±3.68 % in guava, and 15.19±3.91 % in banana, respectively). Interspecific larval competition did not impacted the pupal mass and larval stage duration whatever the density of larvae within the fruit.

Conclusion: The results highlight that in all situations with direct interaction, i.e., competition for resources (larvae) or access to oviposition sites (adults) *B. dorsalis* wins. Those results are in accordance of previous field studies showing higher numbers of *B. dorsalis* in fruits compared to *C. malgassa*.

Keywords: larval and adult competition, Malagasy fruit fly, oriental fruit fly

Poster: Biology, Ecology, Physiology and Behavior

Interspecific competition between an old resident and a newcomer: Is *Rhagoletis cerasi* facing a competitive displacement by *Drosophila suzukii*?

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Background: *Drosophila suzukii* (Diptera: Drosophilidae), also known as the spotted wing Drosophila (SWD) is a polyphagous pest of small and soft fruit of high commercial value, including sweet cherries. The European cherry fruit fly, *Rhagoletis cerasi* (Diptera: Tephritidae) is an oligophagous pest, considered so far as the most important pest of sweet cherries. During the last decade *D. suzukii* has invaded and established in many regions of Asian, Europe and the Americas, while at the same time *R. cerasi* has spread and established in USA and Canada. Co-existence of the two pests in the field and simultaneous infestation of the fruit are already observed in several cherry producing areas. Interspecific competition and even competitive displacement incidents have been observed between polyphagous tephritids, but there is no available information regarding the interaction of tephritids and *D. suzukii*. We investigated possible interspecific competition between *R. cerasi* and *D. suzukii* at adult and larval stage using sweet-cherry as a host.

Methods: Sweet-cherry fruit were individually offered to gravid, mated females of *D. suzukii* or *R. cerasi*. A minimum of 50 fruit from each cultivar were offered for oviposition (a) only to *D. suzukii*, (b) only to *R. cerasi*, (c) simultaneously to *D. suzukii* and *R. cerasi*, and (d) first to *R. cerasi* and three days later to *D. suzukii*. The percentage of sweet-cherry infestation and the number of progeny that concluded larval development and pupated were assessed under controlled laboratory conditions, in five sweet-cherry cultivars.

Results: Our results showed that *D. suzukii* females achieved higher percentages of infestation than *R. cerasi*, in all five cultivars tested. Infestation levels from *R. cerasi* decreased when sweet cherries were offered simultaneously to both pests than when offered to each one separately, in three out of five cultivars. A three-day earlier oviposition of *R. cerasi* before offering the same fruit to *D. suzukii*, led to lower infestation levels from the latter. More *D. suzukii* progeny emerged from infested cherries than *R. cerasi*, adjusting for cherry cultivar and competition between the two pests.

Conclusion: Our findings demonstrate that interspecific competition during female reproduction, as well as during larval development negatively affects infestation levels by both pests in sweet-cherry cultivars. *Rhagoletis cerasi* immatures may face stressful interactions with *D. suzukii* counterparts during their development and therefore pupation decreases in notable levels. The data generated in our study provide useful information to understand the coexistence of the two pests in sweet-cherry orchards.

Keywords: spotted wing Drosophila, European cherry fruit fly, sweet-cherry infestation, larval development, progeny, *Prunus avium*,

Poster: Biology, Ecology, Physiology and Behavior

Interspecific mating of tephritid fruit flies (Diptera: Tephritidae) and the implication for sterile insect technique

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Background: The emerging international trade for horticulture commodities poses a high risk of tephritid pest's expansion worldwide. The melon fly (*Zeugodacus cucurbitae* (Coquillett)), pumpkin fruit fly (*Zeugodacus tau* (Walker)), and oriental fruit fly (*Bactrocera dorsalis* Hendel) are important quarantine pests with partially overlapping host ranges. The increasing number of tephritid pest's invasion has raised the concern of the potential importance of interspecific competition among different tephritid pests.
Methods: we reported the potential use of the novel cross-species sterile insect technique (CS-SIT) in laboratory bioassays. The reproductive interference of these three tephritids caused by interspecific mating showed that *Z. cucurbitae* males form successful mating pairs with *Z. tau* females and vice versa for *Z. tau* in the choice-mating tests. Interspecific mating between male *Z. cucurbitae* and female *Z. tau* resulted in a significant reduction in the mating success of *Z. tau*.
Results: The diel periodicity of *Z. cucurbitae* showed that these males initiated calling behaviors earlier than the *Z. tau* males, which resulted in the *Z. cucurbitae* having more chances to mate with *Z. tau* females. Even though the males of neither species of *Zeugodacus* could compete against *B. dorsalis* males for *B. dorsalis* females, the presence of *Zeugodacus* males reduced the mating success for *B. dorsalis*. Few incidents of interspecific mating were recorded between the *Zeugodacus* males and *B. dorsalis* females at the peak or after the peak of the mating hour.
Conclusion: These findings provide circumstantial evidence for reproductive interference among the three tephritids. The results from this study indicated the potential application value for CS-SIT. Reproductive interference between *Z. cucurbitae* and *Z. tau* may provide the first line of defense against *Z. tau* in areas where *Z. cucurbitae* is implemented

Keywords: Tephritidae; mating; reproductive interference; SIT

Poster: Biology, Ecology, Physiology and Behavior

Limited adaptive capacity of climate stress resistance traits in an invasive agricultural pest

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Background: Adaptive evolutionary potential of climatic stress resistance traits in invasive agricultural pests are rarely investigated but central to forecasting the impacts of climate change and their interactions with biological invasions. In invasive true fruit flies (Diptera: Tephritidae), water balance and thermal tolerances are key traits defining their fundamental niche and thought to contribute to their invasion success. A recently invasive, polyphagous pest, *Bactrocera dorsalis*, swept across Africa from Asia in under a decade and established in a range of environments. A profound capacity for adapting to novel conditions was proposed as an underlying mechanism for this rapid spread.

Methods: To test this notion, we measured desiccation-related traits (e.g., survival time, water loss rates, and size-related metrics of body water content etc.), and heat and cold stress resistance in 1147 flies consisting of 102 paternal lines, where the male parent was known and all offspring in a line were half siblings. We estimated whether climate stress traits are driven by underlying genetic correlations using animal quantitative genetic models. The animal model is a mixed-effects model where additive genetic variance (an independent effect on a phenotype causing deviation from the mean phenotype) for each individual serves as random effect in the model. We analysed the measured traits, obtaining estimates for additive genetic variance (VA) and maternal effects (VM) by investigating either individual or maternal line as random effects in the model. We included initial mass as a fixed effect to determine whether mass has a significant effect on the genetic variance. Lastly, we assessed narrow sense heritability (h^2) to describe the fraction of the phenotypic variance attributed to additive genetic variance.

Results: We show that desiccation-related and heat-and-cold stress resistance traits have low, but significant additive and maternal genetic variance. Mass only had a significant effect on heat knockdown time. Narrow sense heritability was low ($h^2 < 0.393$) except for mass traits associated with desiccation and chill coma recovery time ($h^2 > 0.6$).

Conclusion: *Bactrocera dorsalis* shows low capacity for genetic adaptation in key stress resistance traits and have highly labile body sizes which may also translate to an adaptive fitness advantage when occupying novel environments. As such, we suggest that it is the combination of multiple small mechanisms such as plasticity, adaptive capacity, broad host range, reasonable thermal niche, and high morphological adaptive capacity which directly leads to greater fitness. This would include survival of desiccating or low resource conditions, as estimated here, that give the species an invasion and evolutionary fitness advantage.

Keywords: *Bactrocera dorsalis*, thermal performance, invasive species, agricultural pest

Poster: Biology, Ecology, Physiology and Behavior

Low fruit infestation rate of the world's most polyphagous pest of fruits *Bactrocera dorsalis* (Tephritidae) on the tropical island of Mayotte, despite a long-standing invasion

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Background: The Oriental fruit fly (*Bactrocera dorsalis*), endemic to the Indo-Asian region is one of the world's most invasive and polyphagous pest on fruits and vegetables across the world. This species is extremely polyphagous with more than 500 host plants recorded. In Mayotte, B. dorsalis was recorded for the first time in 2007 but its host range, infestation rate or interactions with other species was not studied.
Methods: In order to identify its impact on the island, its host range and interactions with other resident species was assessed during two field campaigns from January 2012 to January 2014 and from April 2019 to July 2021. We regularly collected fruits all across the island, in cultivated and non-cultivated areas, and compared the infestation rates between the two periods.
Results: We detected six fruit fly species, including the common species *Dacus ciliatus*, *Neoceratitis cyanescens*, *Ceratitis capitata* and *B. dorsalis* and two rarer species *Dacus etiennellus* and *Trirhithrum nigerrimum*. The more surprising result was the low occurrence of *B. dorsalis* with only 7 host plant species identified over the 84 plant species collected in our field sampling. Moreover, infestation rates were low for these species, including for species considered as the major host plants of *B. dorsalis*, such as mango (3.68 flies/kg), guava (12.67 flies/kg) or Indian almond (0.97 flies/kg).
Conclusion: In Mayotte, the infestation rate of *B. dorsalis* was weak compared to other regions, including in Indian Ocean where infestation rates of mango were for example of 38.4 flies/kg in La Réunion or 16.0 flies/kg in Comoros. Different hypotheses are proposed to explain this surprising low infestation rate and narrower host range in Mayotte compared to other invaded areas.

Keywords: Mayotte, Indian Ocean, host range, tephritid diversity

Poster: Biology, Ecology, Physiology and Behavior

Mating with a sterile Mexican fruit fly male reduces female investment in host marking pheromone

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Background: The Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae) is a major pest of mangoes and citrus in Mexico. It is currently controlled using the Sterile Insect Technique, where males are mass-reared, sterilized and released in affected areas. When a female mates with a sterile male she will not have fertile sperm for egg laying. *Anastrepha ludens* females will oviposit after mating, and then will deposit a Host Marking Pheromone (HMP) on the fruit which deters other females from laying eggs. However, we know very little on the effects of mating on HMP behaviour, or how mating with a sterile male differs from mating with a wild male. Differences between sterile and wild males may be due to irradiation or mass-rearing.

Methods: We counted the number of sperm in females mated with wild, sterile or mass-reared fertile males of the bisexual or the GSS strain of *A. ludens* (Tapachula-7). We also measured the size of the male accessory glands (a reproductive tissue) between sterile and wild males. Using videos we measured the time spent host marking on artificial ovipositing devices (green agar spheres), and then counted the number of eggs laid when females mated with a wild or sterile male.

Results: Females that mate with sterile males will receive fewer sperm than females mating with a wild male. Surprisingly, this decrease in sperm is more related to the effects of mass-rearing than to irradiation. We also found differences in male accessory glands (MAGs) between sterile and wild males. When females mated with sterile males, they layed the same amount of eggs as females mating with wild males, however they spent less time depositing HMP than females mating with a wild male. Unmated females lay fewer eggs than mated females, but unmated females will also deposit HMP. Differences in HM behaviour were not related to sperm amount or to receiving MAG fluid.

Conclusion: When females mate with a sterile male she receives fewer sperm and probably a deficient ejaculate, but this did not directly impact female investment in HMP. Females mating with sterile males lay similar amounts of eggs as females mating with wild males but they decreased time spent delivering the HMP, suggesting that females are detecting male condition. Unmated females deposit HMP, thus receiving an ejaculate does not trigger HM behaviour. We are only now beginning to understand how HM behaviour in females can be influenced by female and male condition.

Keywords: Host, Mexican fruit flies, behavior, oviposition

Poster: Biology, Ecology, Physiology and Behavior

Metabolic effect of phytochemical lure in *Bactrocera jarvisi* and *Bactrocera tryoni*

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Background: Plant produced phytochemicals such as raspberry ketone (RK) and zingerone (ZG) attract males of some *Bactrocera* fruit flies as pollinators. Rather than gaining a caloric food reward, males that ingest the lure experience a metabolic boost that can accelerate sexual development of immature males, elevate sexual performance of mature males, and may also have broader impacts on immune function. Mature males of *Bactrocera tryoni*, are attracted to RK, while immature and mature males of *Bactrocera jarvisi* are attracted to ZG. However, almost no information is available on how the lure is metabolized, which downstream biochemical pathways are triggered and for how long after lure ingestion. I will study the effects of phytochemical lure ingestion on the metabolism and physiology of immature and mature males of *B. tryoni* and *B. jarvisi*.

Methods: To gain insights to the metabolic effects of lure feeding in sexually immature *B. tryoni* and *B. jarvisi* males, transcriptomic changes induced by lure ingestion will be assessed by comparing lure-fed males with control males. To identify differential gene expression, reproductive structures that are associated with mating activities, including testes, accessory glands, apodeme and rectal gland, will be dissected for transcriptomic data analysis. To gain insights to metabolic effects of lure feeding in sexually mature *B. tryoni* and *B. jarvisi* males and links to mating and immune function, mature males will be provided access to RK and ZG, respectively, and compared with control males. As with immature males, reproductive structures associated with mating activities will be used for transcriptomic analysis. To test the effect of lure ingestion in immune function, lure-fed and control males will be injected with a solution of *Serratia marcescens*, heat-killed bacteria, PBS or not injected. To assess changes in gene expression related to immune response, transcriptomics will be performed on the dissected fat bodies and proteomics on the haemolymph of males from each experimental group.

Conclusions: This research will address signalling mechanisms, downstream secondary metabolites induced, secondary pathways triggered, and biological consequences of phytochemical lure ingestion by *Bactrocera* fruit flies. An in-depth metabolic study of how these lures affect biochemical pathways is essential to understand the nature and implications of lure-induced metabolic boosts that are important in fruit fly biology and management.

Keywords: *Bactrocera jarvisi*, *Bactrocera tryoni*, Transcriptomics, Proteomics, Mating, Immune challenge, Sexual maturation

Poster: Biology, Ecology, Physiology and Behavior

Novel approaches to gain insights in early detection of low *Ceratitis capitata* populations

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Background: The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Diptera: Tephritidae), threatens the fruit production and trading globally. Despite direct damage in fruit, the presence of medfly in an area may impede fruit trading and impose strict quarantine regulations. Detection of a low-density population of medfly (at early stages of invasion or early in the season in temperate areas) is challenging. In the frameworks of the Horizon 2020 funded project FF-IPM "In-silico boosted, pest prevention and off-season focused IPM against new and emerging fruit flies" we attempted to address the early detection of medfly populations following novel release recapture studies in central Greece, and elaborative trapping efforts in an area that is marginal for the establishment of the pest in northern Greece.

Methods*:* Trapping stations consisted of one plastic McPhail and one Jackson trap baited with food attractant and trimedlure respectively, were established in both experimental approaches. Five trapping stations were deployed in each of four plots (one km^2) that had been established in suburban areas of central Greece. Wildish adults, marked with a fluorescent dye were released from a randomly chosen spot within each plot at two population densities. Recapture efforts lasted at least a month. To detect wild medfly populations in a marginal for its establishment area, the main deciduous fruit production area central Macedonia, Greece, during two growing seasons, the area divided in 50 plots (25 km^2 each) and 25 of them were randomly selected to establish trapping stations. Traps were checked every week during the fruit growing season.

Results: No adults were detected from the low population density (10 males and 10 females per one km2). At the increased density (150 males and 150 females) a proportion of 1.2% adults were recaptured consisting mainly of males (72% of the total captured). Proportion of traps detecting adults was 35%. Recapture rates seems to be independent of the distance between the release and the capture points. The first adults detected at the end of July and at the end of August during 2021 and 2022 respectively, in Macedonia. By the end of both growing seasons 27 out of 37 trapping stations (72%) detected at least one adult. Females were 55% less likely to be detected than males.

Conclusion: Our results reveal that the probability of detecting adult medflies under low population densities is extremely low, even when a dense trapping grid of the best available traps are considered. Low density populations of medfly are scattered in central Macedonia, but the probability of detecting them before August is extremely low.

Keywords: Mediterranean fruit fly, detection efficacy, low- density, release- recapture, trapping, distribution

Poster: Biology, Ecology, Physiology and Behavior

Oviposition Preference of Fruit Flies (*Bactrocera dorsalis* Hendel) on Pondoh Snake Fruits (*Salacca Edulis* Reinw) at Different Levels of Maturity

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Background. Pondoh snake fruit is a mainstay of Indonesian exports, many requests because of its sweet - sour taste and fragrant smell. In the development of pondoh snake fruit exports to foreign countries, there are obstacles, namely the attack of *Bactrocera dorsalis* fruit flies, resulting in rejection from importing countries. Information about the maturity level of exported pondoh snake fruit that can be free from fruit flies is not widely available so it is necessary to test the oviposition preference of *B.dorsalis* fruit flies on snake fruit with several levels of maturity and also to know the life cycle of *B.dorsalis* in pondoh snake fruit. The right level of fruit maturity can be a recommendation for farmers to harvest snake fruit at the right time so that yield losses due to fruit fly attacks can be reduced .

Methode. Testing the level of hardness of salak fruit was carried out using a penetrometer at three levels of hardness, namely 50%, 60% and 70%. Observing the oviposition preference of *B.dorsalis* fruit fly on pondoh snake fruit by observing the number of eggs and percentage of oviposition of fruit flies by *choice method* and *non-choice method*. Observations were made on the life cycle of the *B.dorsalis* fruit fly in pondoh snake fruit.

Results. From observations, that the higher the maturity level of pondoh snake fruit, the value of hardness is lower and the percentage of oviposition preference and the average number of eggs is getting higher**.** In *the choice method*, the results obtained at the maturity level of 50 %, the average percentage of oviposition was 26.6 % and the average number of eggs were 40 eggs. At the maturity level of 60 % obtained an average percentage was 28.3% with an average number of eggs were 42.6 eggs. At the maturity level of 70 %, the average percentage of oviposition was 44.9 % and the average number of eggs were 67.8 eggs. In *the non-choice method*, the results obtained at the maturity level of 50 %, the average percentage of oviposition was 29.7% and the number the average eggs were 39 eggs. At the maturity level of 60 % obtained an average percentage was 31.1 % with an average number of eggs were 40 eggs. At the maturity level of 70 %, the average percentage of oviposition was 51.3% and the average number of eggs were 39.1 eggs. *B. dorsalis* fruit fly prefers to oviposition on softer fruit

Conclussions The oviposition preference of *B.dorsalis* fruit fly at the maturity level of pondoh snake fruit 50 %, 60 % and 70 %, shows the results that the 70 % maturity level has the highest preference level, it can be seen from the oviposition percentage and the highest average number of eggs, both in the method choice or non-choice. *B.dorsalis* fruit flies were able to lay their eggs at the 3 (three) levels of maturity of the pondoh snake fruit and did not show significantly different results. *B. dorsalis* fruit fly has a perfect life cycle in pondoh snake fruit which lasts for 20-25 days.

Key words : Pondoh snake fruit, *Bactrocera dorsalis* fruit fly, Preference, Oviposition, Fruit maturity level, Life cycle.

Poster: Biology, Ecology, Physiology and Behavior

Phylogeographic structure of the oriental fruit fly, *Bactrocera dorsalis*: from the mitochondrial COI gene to nuclear SNPs

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Background: Since its first report in Kenya in 2003, the oriental fruit fly, *Bactrocera dorsalis* (Diptera, Tephritidae), native to tropical and subtropical Asia, is now present throughout sub-Saharan Africa. The Indian Ocean and Oceania have also been invaded during the last decades. In invaded regions, *B. dorsalis* has emerged as a major pest of fruit and vegetable crops causing extensive damage and losses, and affecting the crop trade, especially mango. Although this information is important for assessing the risk of introduction of the species into a new area, to date, the origins of these invasion events are not well identified. Previous studies used traditional genetic markers, such as the mitochondrial cytochrome oxidase I (COI) gene or a dozen of microsatellites, which failed to detect genetic differentiation at spatial scales fine enough to inform on the invasion history of *B. dorsalis.* In this context, our objective was twofold: first, to develop and validate thousands of single nucleotide polymorphisms (SNPs); second, to inform on the global spatial structure of *B. dorsalis* and address the possibility of mitonuclear discordance.
Methods: We first collected 308 *B. dorsalis* specimens which were representative of both the native range (147 specimens distributed in 13 countries from the Oriental biogeographical region) and the invasion range (161 specimens distributed in 22 countries from the Afrotropical, Australasian and Madagascan regions). We then conducted restriction site associated DNA sequencing (RAD-Seq) using the BestRAD approach on a subset of 86 specimens. We analysed the genetic diversity and structure of *B. dorsalis* at the produced nuclear SNPs. Finally, we confronted this data to mitochondrial information at the standard COI barcoding region produced on the 308 specimens.
Results: We successfully identified a large number of high-quality SNPs and showed a high resolution of the phylogeographic structure. We also confirmed the limitations of the COI gene as a useful genetic marker in particular because of a lack of genetic structure within biogeographic areas, a lack of genetic differentiation with some closely-related cryptic species, and the occurrence of mitochondrial DNA introgression.
Conclusion: We developed a new high-throughput molecular tool that showed promise to characterize the invasion pathways of *B. dorsalis* across the world and to assign European incursions to a population of origin. We also invalidated the mitochondrial COI gene as a useful genetic marker for global spatial structure of *B. dorsalis*.

Keywords: Oriental fruit fly, *Bactrocera dorsalis*, Invasive pest, Genetic structure, High-throughput sequencing, mtDNA, RAD-sequencing

Poster: Biology, Ecology, Physiology and Behavior

Phytochemical lure ingestion in males of *Bactrocera* fruit flies: benefits and costs of a metabolic boost

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Background: Tephritid fruit flies are one of the world's most destructive pests of fruits and vegetables. *Bactrocera tryoni* is Eastern Australia's most damaging fruit fly (>100 hosts), and *Bactrocera jarvisi* is a moderate pest in Northern Australia (83 hosts). Phenylbutanoids such as raspberry ketone (RK) and zingerone (ZG) are volatile compounds commonly found in flowers that attract male fruit flies as pollinators. After ingesting phytochemicals, fruit flies gain a metabolic boost, produce a more potent sex pheromone, and have increased mating competitiveness. But these benefits may be countered somewhat by physiological costs. Our project explores both benefits and costs associated with the metabolic boost to immature and mature males produced by ingestion of RK in *B. tryoni* and ZG in *B. jarvisi*.

Methods: The benefits of accelerated sexual development in immature males will be documented by providing access to the phytochemicals and assessing size and development of reproductive organs at different ages. Potential costs in immature males will be determined by comparing longevity of phytochemical fed and unfed males. The benefits of metabolic boost in mature males will be investigated by measuring mating performance and immune response. Mating performance will be assessed through competitive mating success, production of volatile pheromone and cuticular compounds, attraction of females, and analysis of calling songs. Immune response benefits will be measured by comparing survival of phytochemical fed and unfed males injected with pathogenic bacteria, heat-killed bacteria, or PBS solution, or received no injection. The costs of lure ingestion in mature males will be measured through longevity, survival under starvation and desiccation stress, activity levels, fat reserves, and yeast/sugar intake.

Poster: Biology, Ecology, Physiology and Behavior

Predator aversion as a benefit of raspberry ketone consumption in *Bactrocera tryoni*

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Background: Males of many dacine fruit flies feed on biologically active phytochemicals, including raspberry ketone, methyl eugenol, and zingerone, and gain substantial benefits in mating success. Phytochemical feeding may also affect fruit fly interactions with predators. However, the effect raspberry ketone-feeding has on the interaction of *Bactrocera tryoni*, the Queensland fruit fly, and its predators is unknown.
Methods: To assess potential antipredator function of raspberry ketone feeding, we compared responses of a jumping spider predator, *Opisthoncus quadratarius*, to male *B. tryoni* that had (RK+) or had not recently fed on raspberry ketone (RK-). To assess the possibility that through experience spiders learn to more effectively handle RK+ flies we compared responses of naïve (no experience of fruit flies), RK in-experienced (experienced with RK- flies for 30 days) and RK experienced (experienced with RK+ flies for 30 days) spiders. First, the number of successful captures of RK+ or RK- flies by naïve, in-experienced and experienced spiders was enumerated. Second, we assessed whether the experienced spiders retained tactics used for capturing RK+ flies by enumarating capture probability of RK- flies 3, 15 and 30 days after their last experience with an RK+ fly.
Results: We found that after feeding on raspberry ketone male *B. tryoni* gain protection from naïve *O. quadratarius.* However, when naïve spiders were repeatedly provided raspberry ketone-fed flies as prey, they greatly increased their hunting success. Observations of hunting behaviour suggest that the spiders learned to deploy attack from in front of the fly, an approach that is commonly used by jumping spiders to handle noxious prey such as ants. This change in predatory behaviour was not permanent, as after subsequently being repeatedly provided flies that had not been fed raspberry ketone capture success returned to levels similar to those of naïve spiders.
Conclusion: Our findings provide a new dimension to understanding the biology of raspberry ketone feeding by male *B. tryoni* and also highlight a role for learning in the response of a generalist jumping spider predator.

Keywords: Queensland fruit fly, predator, phytochemicals, feeding, raspberry ketone

**Present address*

Poster: Biology, Ecology, Physiology and Behavior

Rapid evolution of a cold stress cline in Mediterranean fruit fly

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Background: The Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), holds an impressive record of successful invasion events promoted by globalization in fruit trade and human mobility. In recent years the species is frequently detected in cooler temperate areas of the Northern Hemisphere. Cold tolerance of *C. capitata* seems to be a crucial feature that promotes population establishment and hence invasion success. The importance of rapid evolution of traits and their phenotypic plasticity, or alternative processes, in facilitating this invasion is however poorly determined.

Methods: We determined: (a) the supercooling capacity (SCP) of immature stages and adults as a measure of cold tolerance, and (b) the acute cold stress survival of adults of *C. capitata* from populations originated from a wide geographical range in the Northern Hemisphere: Austria (Vienna), Croatia (Zaton), Greece (Thessaloniki, Volos, Chania, Heraklion), and Israel (South Arava). To assess the phenotypic plastic capacity in these populations, (c) the effect of acclimation to low temperatures on acute cold stress survival in adults was also examined.

Results: There was marked geographical variation in the SCP of larvae with those originated from Thessaloniki (Greece) and Zaton (Croatia) expressing the highest and the lowest SCP values, respectively. Population and age had significant effects on the SCP of pupae. Young pupae expressed higher SCP compared to old ones, regardless of the population origin. Finally, the SCP of adults was not affected by either population or sex. Acclimation at low temperatures was positively related with survival to acute cold stress. Adults from the warmer environment of South Arava (Israel) were less tolerant of acute cold stress compared with those from Heraklion and Thessaloniki. Plastic responses to cold acclimation were population specific with S. Arava population expressing lower levels compared to the two Greek populations.

Conclusion: Northward range expansions have resulted in the rapid evolution of a cold tolerance cline, even among closely-related populations or nearby localities, or despite high gene flow among populations, that correlate with low temperatures in the region. These results set the stage for asking questions regarding the evolutionary adaptive processes that facilitate range expansions of *C. capitata* into cooler temperate areas of Europe.

Keywords: *Ceratitis capitata*, supercooling, cold response, cold treatment

Poster: Biology, Ecology, Physiology and Behavior

Seasonal Abundance and Infestation of Fruit Flies on Cucumber in Mozambique

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Background: Fruit flies (Diptera: Tephritidae) are pests of economic importance in cucurbits due to the enormous damage they cause directly, and indirectly because of restricted access to export markets. However, information on the abundance and infestation of fruit flies in cucurbits is scarce for Mozambique. Thus, the present study was conducted with the objective of assessing the abundance of fruit flies and infestation on cucurbits, in particular cucumber.

Methods: The study was carried out in the provinces of Maputo and Manica from March 2020 to March 2022. Ten fields were selected containing cucumber. In each field, three Tephritraps were installed with different attractants (cuelure, biolure and zingerone). The traps were inspected and emptied every week. To determine the incidence (number of infested fruits versus total number) and infestation rate (number of flies per kg), 10 fruits were randomly collected per field (every months). The fruits were weighed individually and placed in bowls for the emergence of fruit fly pupae and adults. All emerging insects were preserved in 70% alcohol and identified. The statistical package STATA 15 was used.

Results: 28 species were collected by trapping, of which 15 species in Maputo and 23 species in Manica belonging to the genus *Bactrocera* (1), *Ceratitis* (5), *Dacus* (20), *Zeugodacus* (1) and *Perilampsis* (1). *Dacus frontalis* (37%) and *D. bivittatus* (35%) were the most abundant in Maputo and *D. bivittatus* (69%) and *D. punctatifrons* (21%) were the most abundant in Manica. The invasive species *Zeugodacus cucurbitae* was collected only once in Manica. Thirteen fruit fly species were reared from cucumber. There was no significant difference observed in incidence between Maputo (30%) and Manica (44%). Also, no significant difference was observed in infestation rate between both provinces (39 and 44 pupae/kg respectively in Maputo and Manica); *Dacus ciliatus* was the most abundant fly in Maputo with about 93% of all emerged flies. The infestation rate showed the highest indices with 27,61 adults/kg in Maputo compared to Manica (1,07 adults/kg). Contrary, in Manica *D. bivittatus* was the most abundant with about 68% and showed the highest indices with 32,63 adults/kg than Maputo (0,21 adults/kg).

Conclusion: Cucumbers are being infested by fruit flies in Maputo and Manica province, causing a loss of quantity and quality. *Dacus ciliatus* and *D. bivittatus* are the most economically important species in Maputo and Manica respectively. Therefore, the development of integrated fly management strategies to reduce yield losses in cucurbit crops is recommended.

Keywords: *Fruit flies, abundance, infestation, Dacus ciliatus, Dacus bivittatus*

Poster: Biology, Ecology, Physiology and Behavior

Seasonal reproductive physiology of Queensland fruit fly, *Bactrocera tryoni*

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Background: Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) displays a distinct seasonal phenological pattern, with low abundance during the "overwintering period" (winter or dry season) and a rapid increase in spring (late August/early September). This pattern has been documented throughout its range, including in sub-tropical and tropical regions where temperature is not limiting. Longevity has also been found to vary depending on season, with early spring and early autumn flies relatively short-lived, while late autumn and late winter populations were long-lived.

Methods: Studies were undertaken to examine the seasonal reproductive physiology of female *B. tryoni* using individuals from a colony established from wild-collected infested fruit and maintained outdoors (Redlands, south-east QLD, Australia). The reproductive capacity of ageing, overwintering F1 females was studied between mid-June and the end of August in terms of maturation status, mating status, number of matured eggs and presence of resorbed eggs. Additionally, the reproductive potential of females from successive generations was assessed every ca. two to three months between June and March. Assessments were made of fecundity, maturation status, mating status and number of matured eggs.

Results: The reproductive potential of females was significantly affected by the time of year. During June none of the overwintering females were reproductively active, whereas by the end of August the majority were reproductively active and capable of producing eggs, despite being old (14 weeks). Observations of young females at various timepoints throughout the year demonstrated that their reproductive potential was lowest in June/July and highest in November/December.

Conclusion: Trials demonstrated that females' reproductive capacity decreased during the "overwintering period", despite temperature not being limiting, and peaked during summer. Overwintering, ageing females were able to produce viable eggs in spring and were therefore able to contribute to the spring population peak. We theorise that the low reproductive capacity in the winter months may be linked to increased longevity, while conversely the peak reproductive activity in summer may result in reduced longevity.

Keywords: Queensland fruit fly, *Bactrocera tryoni*, reproductive physiology, overwintering, phenology, population dynamics

Poster: Biology, Ecology, Physiology and Behavior

Sequential invasions by fruit flies (Diptera: Tephritidae) in Pacific and Indian Ocean islands

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Background: Numerous cases of biological invasions have been observed in this family around the world despite serious and strong quarantine procedures. The aim of our study was to examine the cases of Tephritidae invasions across island systems in order to determine whether they follow a hierarchical mode of invasion.

Methods: We reviewed the literature on factors and mechanisms driving invasion sequences in Pacific and Southwest Indian Ocean islands and gathered every record of invasion by a polyphagous tephritid in island groups. From invasion date or period, we defined an invasion link when a new fruit fly established on an island where another polyphagous tephritid is already resident (that was indigenous or a previous invader).

Results: Across surveyed islands, we documented 67 invasion links, involving 24 tephritid species. All invasion links were directional, i.e., they involved a series of invasions by invaders that were closely related to a resident species but were increasingly more competitive. These sequential establishments of species are driven by interspecific competition between resident and exotic species but are also influenced by history, routes, and flows of commercial exchanges and the bridgehead effect.

Conclusion: This information should be used to improve biosecurity measures. Interactions between trade flow, invasive routes, and the presence of invasive and resident species should be integrated into large-scale studies.

Keywords: biosecurity, exotic species, human-mediated dispersal, interspecific competition, patterns of invasion

Poster: Biology, Ecology, Physiology and Behavior

Sexual behavior of *Anastrepha obliqua* (Diptera: Tephritidae): Does laboratory domestication negatively impact male courtship behavior?

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Background: Laboratory colonization of insects is essential for thorough investigations on their physiology, ecology, and management. The ability to rear large numbers of insects is particularly important for species of economic importance, such as tephritid fruit flies, because their management often rely on knowledge of behavioural aspects associated with feeding and reproduction. It is well known that, although essential, insect domestication may lead to deleterious effects on sexual behavior, an important aspect for pest control strategies based on reproductive aspects to be effective. This is particularly important for the sterile insect technique (SIT).

Methods: In this work, we analyzed the influence of laboratory colonization on courtship behavior of *Anastrepha obliqua*. We compared the sequence and frequency of the behavioral units displayed by laboratory reared and wild collected insects. We also analyzed the temporal pattern of male pheromone emission from both strains.

Results: The observed courtship units were divided into nine categories to define the ethogram of the specie. A behavioral sequence leading to copulation success is composed of Attempt (AT) followed by Mating (MT). There is no difference in the courtship behavior units displayed between the wild and laboratory strains of *A. obliqua*. Both strains of *A. obliqua* have shown the behavioral unit Attempt (AT) as a precedent to Mating (MT) being observed difference between the two strains only in the execution of the behavioral units that precede Attempt (AT). GLM analysis showed differences in the frequencies of a few behavioral units between the two strains. The analysis also showed similarities in the temporal pattern of pheromone emission, showing two peaks, in the early morning and the late afternoon, for the two *A. obliqua* strains.

Conclusion: Laboratory colonization does not negatively impact the courtship behavior of *A. obliqua males*.

Keywords: Fruit flies, Sterile Insect Technique, Courtship behavior

Poster: Biology, Ecology, Physiology and Behavior

So close, yet so far: the story of *Ceratitis rosa* and *Ceratitis quilicii* (Diptera: Tephritidae)

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Background: In 2016, *Ceratitis rosa* s.l was divided into two distinct species: *Ceratitis rosa* Karsch and *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio following differences found principally in their genetics and physiology. Morphologically, only males of the two species can be differentiated. Very little is known about the ecology and biology of these flies as separate species. The ecology and biology of the two species were therefore studied in South Africa where both species co-occur.

Methods: Populations and host ranges of the two fruit fly species were quantified through trapping and fruit sampling surveys in eight provinces of South Africa between March 2020 – November 2021 in the late summer, autumn-winter, and spring-summer seasons. Effects of selected environmental and climatic factors on the abundances of the fly species were determined. In laboratory studies, success of immatures (developmental times and survival rates of larvae and pupae), and survival rate and reproduction in adults were determined on five fruit types: guava, mango, peach, mandarin, and orange, under constant optimal temperatures.

Results: *Ceratitis quilicii* was generally more widespread and abundant than *C. rosa* in South Africa. Both species co-occurred only in the north-eastern parts of the country and were more abundant in summer than in winter. Habitat type influenced abundance of the two fly species in areas of co-occurrence: *C. quilicii* dominated in trap catches in the commercial fruit farms whilst in non-commercial environments, the two species were equally abundant. In these non-commercial environments, *C. rosa* dominated in co-infested fruits. Altitude, minimum temperature, and relative humidity influenced the populations of *C. quilicii* but not *C. rosa*. *Ceratitis rosa* was absent in areas where minimum winter temperatures were below 8 °C while *C. quilicii* was present even in areas with minimum temperatures of <1 °C. In laboratory studies, fruit type influenced immature success and immature success was poor on the two citrus fruits for both fly species. Among fruit types, adults of both fly species reared on mango were more fecund, fertile, and lived longer. *Ceratitis rosa* was more fecund and fertile than *C. quilicii* on peach, guava, and mango. On these fruit types, calculated population parameters predicted faster population growth for *C. rosa* than *C. quilicii*.

Conclusion: Despite being close morphologically, there were large differences in the ecology and biology of *C. rosa* and *C. quilicii*. It was clear that abiotic factors played a significant role in the population dynamics of the two species. If abiotic factors are optimal for both species and fruit which are hosts to both species are available, *C. rosa* could outcompete *C. quilicii*.

Keywords: Cape fly, Natal fly, ecology, demography

Poster: Biology, Ecology, Physiology and Behavior

Standardisation of oviposition behaviour of melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae): a pre-requisite for microinjection of embryos

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Background: The melon fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), is a major pest affecting cucurbit crops in various regions of Asia (Dhillon *et al.*, 2005). Sterile insect technique (SIT) is a pest management strategy that involves the mass-rearing of flies rendered infertile males by irradiation. Fitness costs associated with irradiation and SIT efficiency can be improved in precision guided SIT (pgSIT) by using the CRISPR/Cas9 gene editing tool which can create site-specific target gene mutations of flies. The delivery of single guide RNA + Cas9 endonuclease enzyme (ribonucleoprotein complex) by microinjection into embryos is an important step to generate mutants. However, the delivery of the ribonucleoprotein complex needs to be completed in the G_0 embryo cell division phase, preferably within one hour after egg laying to achieve heritable editing. Therefore, the standardization of oviposition conditions is important for the success of pgSIT.

Methods: We reared and maintained *Z. cucurbitae* at 25±1°C, 75±1% relative humidity with 14h:10h L:D photoperiod, and evaluated its preference for four oviposition substrates: cucumber (*Cucumis sativus*), ridge gourd (*Luffa acutangula*), bitter gourd (*Momordica charantia*) and pumpkin (*Cucurbita pepo*). We counted the numbers of eggs laid in different substrates, and assessed the daily egg laying pattern and oviposition period across different fly age groups. For this we prepared small plastic cups (2 cm height, 3 cm diameter) filled with water and covered with parafilm. Then we placed one thin slice (ca. 2-5 mm) of the different substrates on top of each cup (five replicates) and kept these in rearing cages (14 cm height, 11 cm diameter) with 15 males and 15 females for egg collection. Observations were taken from 11 day to 30 days after adult eclosion. All the data were square root transformed and subjected to two-way ANOVA.

Results: Results revealed that cucumber was the most preferred fruit for oviposition, with significantly more eggs collected. The flies were sexually active and laid more eggs from 15 to 25 days after adult eclosion. The flies oviposited significantly more eggs during mid-afternoon (13:00-14:00) than the early morning (09:00-10:00) and late evening (17:00-18:00).

Conclusion: Cucumber is the cheapest fruit and readily available in India throughout the year. It can be used for rearing of melon fly and egg collection for embryo microinjection. A healthy cohort of females aged between 15 to 25 days can be used for harvesting good numbers of eggs. Mid-afternoon is a suitable daytime to collect good number of eggs for embryo microinjections as required for CRISPR/Cas9 based gene editing to achieve greater efficiency by SIT.

Keywords: Melon fly, CRISPR/Cas9, Microinjection, Oviposition

Poster: Biology, Ecology, Physiology and Behavior

Susceptibility of indigenous and introduced olive varieties to olive fruit fly infestation in Croatia

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Background: Olives have been cultivated in the Mediterranean region and in Croatia for more than 2,500 years. Croatia has extremely favourable conditions for olive cultivation, and indigenous varieties and those introduced from Italy are grown on an area of 18,600 hectares.
Olive cultivation, and thus the production of quality olive oil, is limited by pests, the most important of which is the olive fruit fly. Olive varieties differ in their susceptibility to this pest, and 60 varieties are grown in Croatia.
Methods: The susceptibility of two indigenous (Oblica and Istarska bjelica) and four varieties introduced from Italy (Ascolana Tenera, Frantoio, Leccino and Pendolino) to olive fruit fly infestation was investigated at two important cultivation sites in Croatia (Vodnjan and Benkovac). Three trees of each cultivar were selected, and 100 olive fruits were collected from each tree. The fruits were first visually inspected for olive fruit fly infestation and then prepared for pest development monitoring according to a standardised protocol. Pest development was monitored weekly.
Results: The olive grove in Vodnjan had a significantly higher percentage of fruit infested with olive fruit fly. Most flies developed in the fruits of the indigenous Istrian variety Istarska bjelica (145 specimens), while the second most infested variety was the Italian variety Frantoio (119 specimens). The varieties Oblica, Ascolana Tenera and Pendolino had the same number of flies (75 - 79 specimens), and the small-fruited variety Leccino had the lowest number of flies (70 specimens). In the olive grove of Benkovac, the intensity of olive fruit fly infestation was much lower, and in this area the Ascolana Tenera variety was the most frequently infested (59 specimens), followed by Leccino (47 specimens). The varieties Pendolino, Oblica and Istarska bjelica were about equally frequently infested (26 - 39 specimens), and the variety Frantoio was the least infested (12 specimens).
Conclusion: Varieties with large fruits (Ascolana Tenera) and varieties with long ripening period (Istarska bjelica and Frantoio) are more susceptible to olive fruit fly infestation than other varieties. Favourable climatic conditions for olive fruit fly development in the study areas and insufficient pest control measures in Vodnjan also contributed to the heavy infestation.
Cultivation of the varieties Istarska bjelica and Ascolana Tenera is threatened by this pest in the studied areas.

Keywords: *Olea europea* L., *Bactrocera oleae* (Rossi, 1790), cultivar sensitivity

Poster: Biology, Ecology, Physiology and Behavior

Temperature-dependent resting metabolic rate of fertile and sterile oriental fruit flies when fed different diets

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Background: The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is an invasive horticultural pest that has rapidly spread across Africa. While not yet implemented in Africa to manage *B. dorsalis*, the sterile insect technique (SIT) may be a viable control method in some regions. However, sterile males released into the field may perform poorly due to laboratory adaptation, handling and irradiation. Interventions such as pre-release treatment of sterile males with a protein-supplemented diet can boost sterile male reproductive development and mating performance but this may negatively affect lifespan. Underlying all of these traits is energy metabolism, which relies on adequate dietary intake of macronutrients to fuel biochemical processes but may also be damaged by radiation. In this study we evaluated the effects of gamma irradiation and diet on the resting metabolic rate of male *B. dorsalis*.

Methods: We used flow-through respirometry to measure the resting metabolic rate of male flies based on their CO_2 emissions. In this system, 14-day-old adult males of known body weight were placed individually in chambers through which dry, CO_2-free air was passed before being directed to a CO_2 gas analyser. The CO_2 emitted by each fly when inactive was divided by its body weight to calculate a mass-specific estimate of metabolic rate. Males were either fertile or sterile, with sterility being induced by gamma irradiation of pupae at the dark brown eye colour stage with 100 Gy from a cobalt-60 source. From adult emergence, males were fed with either continuous access to sugar only or sugar and hydrolysed yeast. During metabolic rate testing, the temperature was held at 15, 20, 25, 30 or 35˚C.

Results: Temperature significantly affected the resting metabolic rate of male *B. dorsalis*, regardless of sterility or diet, with an approximate doubling for each increase in temperature of 10˚C. The metabolic rate of sugar-fed males was significantly lower than that of males fed sugar and hydrolysed yeast. Sterility had no noticeable effect on metabolic rate.

Conclusion: Our results suggest that gamma irradiation of *B. dorsalis* at the recommended dose and developmental stage does not negatively affect the resting metabolic rate of males. However, the higher metabolic rate of males fed sugar and hydrolysed yeast provides a potential mechanistic basis for their poor survival in the field due to an upregulated rate of living that drives improved mating performance.

Keywords: *Bactrocera dorsalis*, diet, metabolic rate, sterile insect technique

Poster: Biology, Ecology, Physiology and Behavior

The resistance of chili varieties to fruit fly (*Bactrocera dorsalis*) infestation

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Background: Indonesia is known as one of fresh chili producer, but its contribution is lower than the world average production. Chili is potential host for fruit fly (FF) because it planted throughout the year. The estimated chili losses due to FF up to 100%. The objective of this research was to investigate the response of different varieties of chili upon fruit fly infestation trough antixenosis and antibiosis tests.
Methods: Fifty chili varieties belong to four species of *Capsicum* (*Capsicum annuum*, *C. chinense*, *C. frutescens* and *C. pubescence*) selected based on farmer choice for cultivation. Fifty chili varieties (*Capsicum* spp.) were planted in the screen house, subjected to antixenosis and antibiosis tests. **Antixenosis** was conducted by choice and non-choice method. The choice was conducted by putting fifty chili fruits from 50 different varieties and infested by 25 pairs of FF for 48 h and was repeated nine times. A non-choice method was conducted on infested 25 pairs of FF contain five chili fruits represent different variety of chili for 48 h, with 3repetition. Fruit fly oviposition was indicated by the presence of small puncture on the fruit surface. **Antibiosis test** was conducted by inserting three eggs into the fruit pericarp of each chili varieties. The emergence imago was counted every day until 15 days. The antibiosis of FF infestation on chili was calculated using Fitness Index (FI). A total 500 chili fruits were used for this experiment.
Results: In antixenosis test - choice method: The female fruit fly oviposit their eggs only in certain chili. This condition showed by the higher of oviposition deterrent index (ODI) (81.87%). At antixenosis - non-choice method showed that female FF laid their eggs in all varieties of chili. Fruit fly oviposition concentrated on *C. annuum* and *C. chinense* with ODI varied between (11.11 to 99.07)%. In a**ntibiosis test:** Eggs survival of FF in different varieties of chili varied at different value. This experiment found that 9varieties was highly resistant; 22varieties was moderately resistant; 11varieties was low; 5varieties was susceptible, and 3varieties was highly susceptible.
Conclusion: The variety of *Capsicum annuum* more resistant to fruit fly infestation than, *Capsicum frutescense*, *Capsicum chinense* and *Capsicum pubescence* showing by the highest oviposition deterrence index (96.48±4.76/NC) and smallest Fitness Index (0.82±0.80).

Poster: Biology, Ecology, Physiology and Behavior

Unraveling the distribution of *Ceratitis rosa* and *Ceratitis quilicii* (Diptera: Tephritidae) in southern Africa

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Background: *Ceratitis rosa* Karsch and *C. quilicii* De Meyer, Mwatawala & Virgilio are two closely related agricultural pests, belonging to the FARQ complex, whose females are morphologically indistinguishable. *Ceratitis quilicii* was first described in 2016 and until then was considered as a single taxon with *C. rosa*. For this reason, the distributions of these two species are only vaguely known. The project DISPEST, funded by the Belgian Development Cooperation, aims at providing more updated information on the distributions of these two species in South Africa and Mozambique.

Methods: Male vouchers deposited in African and European collections were morphologically reanalysed. Additionally, surveys were carried on between 2020 and 2022 in South Africa and Mozambique. In South Africa, McPhail type traps baited with Enriched Ginger root Oil were set up in 32 sites in eight provinces. In Mozambique, trapping was conducted at 71 sites in the southern, central, and northern provinces. Catches of male specimens were identified using electronic multi-entry keys and confirmed using genomic tools. The 2020-2022 trapping records were then compared with the updated historical records.

Results: In South Africa, *Ceratitis quilicii* was recorded in all provinces except the Northern Cape Province. Relatively high numbers of *C. quilicii* were trapped in the North-West Province compared to the other provinces. *Ceratitis rosa* within South Africa was trapped in only three north-eastern provinces (Limpopo, Mpumalanga, KwaZulu-Natal). In Mozambique, in contrast, *C. quilicii* was only trapped in the Maputo province which is in the south of the country whereas *C. rosa* was widely collected in all other provinces. Relatively higher numbers of *C. rosa* were trapped in Inhambane and Maputo provinces compared to the other provinces.

Conclusion: The results from the recent surveys in southern Africa are largely in agreement with the revised historical records and confirm that *C. rosa* has a wider distribution in Mozambique whilst *C. quilicii* is more widespread in South Africa.
As planned in the framework of the project DISPEST, information on the updated distributions, combined with genetic structuring and landscape modelling data will provide useful indications on optimal pest management strategies for these two agricultural pests.

Keywords: Ceratitis**,** FARQ complex, South Africa, Mozambique

Poster: Biology, Ecology, Physiology and Behavior

What happens when a female mates? Behavioral, transcriptomic and proteomic studies reveal novel phenotypes in the Mexican fruit fly

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Background: The Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae) is a major pest of mangoes and citrus. Within the context of the Sterile Insect Technique, females mating with a sterile male will receive sperm with lethal mutations so they cannot lay fertile eggs. Along with the mutated sperm, females will also receive seminal fluid (SF), including proteins from the male accessory glands (MAGs) and other reproductive tissues. These can affect female postcopulatory responses. Here, we review what we know about *A. ludens* postcopulatory behaviour, in terms of the ejaculate (sperm and SF), female remating, olfactory response and oviposition behaviour, gene expression and proteomics.

Methods: Utilizing behavioural assays, chemical ecology, transcriptomics, and proteomics we have a better understanding of female postcopulatory changes. For behavioural assays we compared females that 1) received only SF using microsurgery to remove the testes or MAG injections into the female thorax, with 2) females that mated with males whose distiphallus had been cut, and only received a mechanical stimulus and no ejaculate, with 3) mated females and 4) unmated females. We evaluated female response to male pheromones and host volatiles in an olfactometer, and female visits and captures in baited traps in field cages. RNA-seq data was compared from mated and unmated males and females, while proteomic data was obtained by subtracting proteins found in mated and unmated females with proteins found in the male reproductive tissues: MAGs, testes and ejaculatory apodeme.

Results: Mating elicits in females: 1) a preferred response to host volatiles over male pheromone, 2) an increase in oviposition, and 3) a sexual refractory period. However, as opposed to other tephritids, these changes are not elicited by SFPs. The mechanical stimulus of mating increases oviposition even if females do not receive an ejaculate. Mating regulates genes, with D. melanogaster orthologues, involved in egg development, pathogenic defence, wound healing, detoxification, musculature and chemosensory perception. MAGs, on the other hand, express genes related to lifespan response to stressful environmental factors, and sugar regulation. Proteomic data revealed proteins in the MAGs transferred during mating involved in the formation of olfactory memory.

Conclusion: Identification of proteins transferred to females during mating may guide future efforts at making reproductive-based control techniques more efficient, such as inducing sterility in males, increasing oviposition in mass-reared females or developing a more efficient olfactory based trap. The function of SFPs in *A. ludens* is still not well-understood, but behavioural, transcriptomic and proteomic data brings us closer to understanding how the male ejaculate changes female phenotypes.

Keywords: Anastrepha, behaviour, oviposition, mating, RNA-seq, proteomics

Poster: Biology, Ecology, Physiology and Behavior

Yeast mediates the interspecific interaction between introduced *Bactrocera dorsalis* and indigenous *Bactrocera minax*

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Background: Host plant-microbe associations mediate interspecific interaction among herbivorous insects. However, this theory has rarely been verified in tephritid fruit flies ecologically. In recent years, we found mixed infestations by the larvae of *Bactrocera minax* and *Bactrocera dorsalis* in citrus orchards which give us opportunity to explore the underlying mechanism. The Chinese citrus fly, *B. minax* is an oligophagous species endemic to China, and female oviposit into green unripe citrus fruits, whereas invasive species *B. dorsalis* is polyphagous, and flies lay eggs in ripe fruits.
Methods: We investigated the occurrence and fruit damage caused by two species in the citrus orchard, and analyzed the response of *B. dorsalis* to the fruits previously infested by *B. minax* (Bm-maggoty fruits). In addition, yeasts from Bm-maggoty fruits were isolated and identified, and the volatiles of yeast and Bm-maggoty fruits were identified by GC-MS. Finally, we assessed the effects of interspecific interaction on the fitness of two tephritid fruit flies through laboratory assays.
Results: After oviposition by *B. minax*, yeast *Pichia kluyveri* were transferred to the citrus fruit, and then proliferated inside; D-limonene released from infested citrus fruits containing *P. kluyveri* was 27 times higher than that in healthy fruits; mature *B. dorsalis* adults were significantly attracted by D-limonene, and thus oviposited into the Bm-maggoty fruits. What's more, interspecific interaction between these two species in the same fruit significantly decreased the number of surviving larvae and pupal weight of *B. dorsalis*, but it had less effect on *B. minax*.
Conclusions: This study suggests, in the interspecific interaction between these two species, *B. minax* occupies the dominant position. This has ecological significance for *B. minax* in terms of consolidating its own niche and inhibiting invasion from exotic species. This is the first case which reveals symbiotic yeast mediating the interaction between *B. minax* and *B. dorsalis* by altering fruit volatiles from the perspective of ecology as well as physiology.

Keywords: Tephritid fruit flies, plant volatiles, interspecific interaction, yeast

Poster: Taxonomy and Morphology

Development of a multi-entry identification key for economically important fruit fly (Diptera: Tephritidae: Dacinae) larvae

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Background: Identification of fruit fly larvae are typically difficult due to the limited morphological characteristics present. However, this is the stage at which fruit flies are intercepted at ports of entry when importing fruits and vegetables. Molecular tools are useful, but DNA analyses take time and are expensive, compared to morphological identifications. This project aims to use available information from literature and our own research to build a multi-entry identification key for thirteen tephritid species, including species complexes, such as the *B. dorsalis* complex and *Ceraitits* FAR complex, that are of economic concern for the European Union.

Methods: Third instar larvae were obtained from a variety of different regions and hosts, including South Africa, La Reunion, India, Spain, China, Greece, Israel, Australia and the IAEA colonies in Austria. Thirteen species or representatives of species groups were obtained, based on availability, including *Ceratitis*, *Dacus*, *Bactrocera* and *Zeugodacus* spp. The cephalopharyngeal skeletons were dissected out, cleared in a 10% NaOH solution, dehydrated with 70-100% alcohol and mounted in Euparal on glass slides. Images of at least 20 larvae/species were captured using a compound microscope fitted with a camera and 400x magnification, measurements taken of 1) distance between ventral apodeme and apical tooth, 2) ventral angle between apical tooth and ventral apodeme, 3) distance between dorsal apodeme and ventral apodeme, 4) ventral apodeme and pre-apical tooth, and 5) apical tooth and pre-apical tooth. The number of tubules in the front spiracles were counted and the position of the spiracles in relation to the cephalic skeleton was noted. Data were analysed using a one-way ANOVA with multiple variables and discriminant analysis with classification functions of the effective principal components.

Results: For development of the multi-entry key and mobile application, the LUCID software was used. A matrix was compiled for 13 species and included 9 characters for which significant inter-specific differentiation was preliminarily detected. The key starts with options for separating Tephritidae larvae from other commonly encountered insect larvae in fruit, including Drosophilidae, Tortricidae, Pyralidae, Nitidulidae and Lonchaeidae. Finally, the key was converted into a mobile application by LUCID, for both Android and Apple devices.

Conclusion: This is the first time a multi-entry key for tephritid larvae of economic significance has been developed. While the characters rely mostly on measurements, it does require some knowledge of dissecting out and treating the mouthparts, so that measurements of specific distances can be made. However, as nothing else exists for this list of species, it will be a valuable tool for enabling non-molecular identifications of fruit fly larval pests in fruit.

Keywords: Morphological identification, *Ceratitis capitata, Bactrocera dorsalis, Bactrocera tryoni, Zeugodacus curcurbitae,* quick detection

Poster: Taxonomy and Morphology

Polyandry is an important reproductive trait of the invasive *Drosophila suzukii*

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Background: The spotted wing drosophila, *Drosophila suzukii* Matsumura, is a pest of soft-skinned fruit native to Eastern Asia which, since 2008, has become a world-wide pest in many Asian, American and European countries. This rapid global spread is supported by several adaptive life history traits such as a high degree of polyphagy. Very important is its ability to overwinter as "reproductively quiescent" long-lived melanized winter morphs characterized by a lack of ovary development during winter and the resumption of ovary development in spring. *Drosophila suzukii* flies possess a high reproductive rate due to a long adult lifespan and high female fecundity. To understand the population dynamics of this fly and develop control management strategies, it is important to evaluate factors underlying such a high reproductive rate. An open question is whether polyandry is a common behaviour in the wild and if so, how does the female use the sperm from multiple matings?
Methods: Females were collected from a North Italian population at the peak of its exponential growth. Paternity analyses were accomplished using SSR informative markers to determine the maternal and offspring genotypes. The remating frequencies were estimarted with the programs GERUD1.0 and GerudSim2. In addition, a Bayesian method was used to estimate parameters of multiple mating and sperm displacement.
Results: A high remating frequency was detected among the wild females of the considered population. Indeed, 83% of the tested families had been sired by more than one male, from a minimum of 2 to a maximum of 5, with an average of 3.25 fathers. Bayesian simulations strongly indicated that females from this population do not exhibit sperm displacement, β, which was estimated to be 0.504 (95% confidence level = 8.502E-05). Thus females use spermatozoa from all their male partners for siring their progeny.
Conclusion: Remating is a common event in the wild and, what is more, females of *D. suzukii*, unlike *D. melanogaster*, store sperm from different males and then use the sperm for fertilization. These are biologically relevant data for interpreting the high reproductive biology of this drosophila species. Remating influences the effective population size and may constitute a critical factor in determining the success of control methods including the SIT.

Keywords: *Drosophila suzukii*, invasive species, polyandry, sperm use

Poster: Taxonomy and Morphology

Exploring the Potential of Wing Shape Variation in Discriminating *Bactrocera dorsalis* and *Bactrocera kandiensis* (Diptera: Tephritidae) using Canonical Variates Analysis.

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Background: Bactrocera dorsalis is the most frightening fruit pest in Asia and *B. kandiensis* is an endemic fruit pest to Sri Lanka. In addition, *B. correcta* and *Zeugodacus cucurbitae* are other commonly recorded fruit fly species in Sri Lanka. There were many taxonomic impediments when discriminating fruit fly species using their wing shape and wing venation patterns, especially due to the cryptic speciation between *Bactrocera dorsalis* and *B. kandiensis.* Hence the present study aimed to differentiate *B. dorsalis* and *B. kandiensis* using an in-group (*B. correcta*) and an out-group (*Z. cucurbitae*) species by applying the wing geometric morphometric (canonical variates) analysis.

Methods: Cumulative fifteen wing landmarks were generated by the software series: tpsUtil64 and tpsdig264 from 240 geographically distinct male fruit fly samples of three species of the genus *Bactrocera*: *B. dorsalis*, *B. kandiensis* and *B. correcta* and one species of the genus *Zeugodacus*: *Z. cucurbitae* were used to analyse the wing shape variation data using canonical variates analysis by the software: SPSSv 17.0. All adult male flies of four species were collected from selected ten field sites using pheromone traps (methyl eugenol) (5 cm diameter, 10 cm height) from March to May 2022. All the calculations were made through a new set of co-ordinate data obtained by generalised Procrustes fit/superimposition for all the individuals in the sample generated by the computer programme: MorphoJv 1.02E.

Results: Degree of variation in wing shape on centroid size (normalized) revealed a significant contribution ($P < 0.0001$) on multiple regression analysis of the sample studied. Ten significant canonical variates (CVs) ($\alpha = 0.05$) resulted from canonical variates analysis on generalized Procrustes superimposition of the sample data. First three canonical variates: CV_1 ($\lambda = 0.001$, $\chi^2 = 1538.626$, $P < 0.001$), CV_2 ($\lambda = 0.005$, $\chi^2 = 1200.255$, $P < 0.001$) and CV_3 ($\lambda = 0.019$, $\chi^2 = 882.428$, $P < 0.001$) contributed to the 71.1% of the variation. Interspecific wing shape variation of the four fruit fly species was observed as separate clusters under the graphical representation of the first three canonical variates respectively and it is independent of their intraspecific variation.

Conclusion: Wing shape variation has the potential in discriminating phenotypically close fruit fly species. The present study validates the applicability of wing shape variation to discriminate between *Bactrocera dorsalis* and *B. kandiensis* through landmark-based geometric-morphometric analysis in taxonomic studies. A consolidated method of the above concept with the molecular techniques in their population management is highly recommended for future studies.

Keywords: *Bactrocera dorsalis*, *B.kandiensis*, Canonical variates analysis, Cryptic speciation, Wing shape variation

Poster: Taxonomy and Morphology

Morphological and molecular characterization of the *Zeugodacus cucurbitae* melon fly in Kurunegala District, Sri Lanka.

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Background: The melon fly (*Zeugudacus cucurbitae*) is one of the prominent pest species of the cucurbit plants in Sri Lanka. So far, the primary tools used for identifying the melon fly species (*Z. cucurbitae*) are a combination of morphological features and the distinctive color pattern of the adult body and wings described in the standard taxonomic guidelines. Drawbacks of field sample collection and delivery methods severely affected the proper identification and were time-consuming. In fact, the exact identification of melon fly species is difficult in large-scale surveillance, and scientist couldn't rely solely on morphological identification. Thus, necessary to identify possible alternative methods like molecular characterization that could be used parallel with the morphological identification method for large-scale surveillances.

Methods: Melon fly samples were collected from different locations of the preselected melon farm in Kurunegala district (7°59'15.3"N and 80°22'12.2" E). Adults were collected from the fly trap with food bait composed of pumpkin juice, peptone, honey, and cooking oil. Mature rotten melons harbouring fruit fly larvae were also collected and reared inside the insectary until adults emerge. The melon fly adults were morphologically identified using standard taxonomic keys under the stereo-type microscope with a magnification of 10x for external examination of the adult and x200 for examination of the larvae. Identification of the melon fly was also performed using a molecular approach. DNA was extracted from the whole body of the melon fly adult and used for the PCR and genetic analysis of the target ITS gene sequence of the melon fly population.

Results: Based on the identified morphological characteristics species was confirmed as *Zeugudacus cucurbitae*. Identified characters included; the size of species medium to large 8.3 - 11mm, width 1.5-2.7 mm, head: antenna with two segments, oral ridges present with 17-23 rows of moderately long, bluntly rounded teeth, elevated anterior spiracles with 16-20 tubules in a single uniform row. Molecular identification based on sequencing data also confirmed the same outcome with 99% similarity for the same species sequences submitted to the GenBank sequence database, and molecular phylogeny reveals the existence of two sibling species within the study samples.

Conclusion: According to the morphological and molecular identification results major melon fly species found within the study samples is *Zeugudacus cucurbitae.* Finding two sibling species from the molecular identification confirms the problem associated with morphological identification. Therefore, a combined identification method could be a better alternative for the large-scale field surveillance to identify the *Z. cucurbitae* species abundance in Sri Lanka, possibly required to perform before implementing target-specific control measures like the Sterile Insect Technique (SIT).

Keywords: *Zeugudacus cucurbitae*, Taxonomic keys, Molecular identification

Poster: Taxonomy and Morphology

Nine years of fruit fly (Diptera: Tephritidae: Dacinae: Dacini) surveys in Bangladesh: a new species, 28 new country records and key to the species

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Background: Until 2013, field research on dacine fruit flies in Bangladesh had mainly focused on studying ecology and developing field control for cucurbit-infesting pest species and systematic field surveys had been very limited to that point. To generate a complete inventory of the economic and other species and provide identification keys to dacine fruit flies in the rural farmland and protected forest areas of Bangladesh, extensive surveys were conducted between 2013 to 2021.
Methods: Traps were baited with three different male lures (cue-lure, methyl eugenol, zingerone) plus a piece of dichlorvos strip to kill trapped flies and propylene glycol solution was used to preserve specimens. Between April 2013 and September 2021, traps were deployed at 625 sites and maintained for 3-7days. Sampled flies were stored in 95% ethanol in a −20°C freezer, to preserve DNA for analysis. All flies were identified to species level by using available keys. For series of selected specimens, DNA was extracted and the Cytochrome C Oxidase I (COI) and Elongation Factor 1-alpha (EF1-alpha) genes were sequenced to help confirm species identifications. Starting in 2021, multilure traps baited with synthetic food lures were also used to collect other species of fruit flies.
Results: Systematic field surveys, ongoing since 2013, have increased the number of Dacine fly species from 6 to 34 in Bangladesh, including a new species named *Zeugodacus madhupuri* Leblanc & Doorenweerd. The highlight among the new records was the detection of *Bactrocera carambolae* Drew & Hancock in Bangladesh, a significant westward range extension of this destructive pest. Other pest species in the country include fruit pests *Bactrocera dorsalis* (Hendel), *B. zonata* (Saunders), *B. correcta* (Bezzi), *B. latifrons* (Hendel), *B. tuberculata* (Bezzi), cucurbit fruit pests *Zeugodacus cucurbitae* (Coquillett), *Z. tau* (Walker), *Z. hochii* (Zia), *Dacus ciliatus* Loew, and *D. longicornis* Wiedemann, and cucurbit flower pests *Zeugodacus caudatus* (Fabricius) and *Z. diversus* (Coquillett). *Bactrocera dorsalis* in Bangladesh exhibits a broad range of scutum and abdomen color pattern variation, similar to populations of synonymous *B. invadens* Drew, Tsuruta and White. An annotated checklist and illustrated key of 34 dacini fruit flies to the species was published in 2021. The deployment of traps baited with synthetic food lures have yielded so far 10 additional species.
Conclusion: The published checklist, distribution maps, lures and host records, and the fully illustrated key to the 34 species in Bangladesh will greatly help entomologists carry out their own monitoring and carry out pest risk analysis for cultivated fruits and vegetables and develop control strategies.

Keywords: Dacini, *Bactrocera*, *Dacus*, *Zeugodacus*, pest species, taxonomy.

Poster: Taxonomy and Morphology

Take a swipe at the fly: fruit fly (Diptera: Tephritidae) identification through mobile applications

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Background: Morphological identification of adult insects traditionally relies on the use of dichotomous keys which are generally not easily accessible and/or problematic to be used by the non-specialist. The lack of easy and readily available tools hampers a swift and timely identification, of crucial importance in detection and surveillance programmes. Contemporary digital and communication technology offers possibilities for the development of alternative identification tools. Within the framework of two projects (EU Horizon 2020 project 818184 FF-IPM; and STDF PG 567 project F³) the development of such tools is envisaged.

Methods: We aim to develop mobile applications that can be downloadable on smartphones, for a number of selected species tailored to the needs of the respective projects. The development starts from existing multi-access (i.e. non-dichotomous) identification keys for frugivorous fruit flies, developed on the Lucid platform. In addition, enhanced high-resolution images will be taken for further annotation and illustration of diagnostic morphological characters. Dedicated datasheets and hyperlinks with relevant information will be added.

Results: Two mobile applications were developed. One application includes 23 target species of the genera *Ceratitis*, *Bactrocera* and *Zeugodacus* that are considered of importance for the EU. The list was compiled after consultation with local NPPO's, EPPO and other potential end-users. A second application comprises 29 major fruit fly pest species of the genera *Ceratitis*, *Trirhithrum*, *Bactrocera* and *Zeugodacus*, occurring in different regions in Africa, including the islands in the western Indian Ocean. Both applications are developed along similar lines with a pre-key allowing selection to genus level, and subsequent keys to targeted species within each genus. The platform used allows for selection of any characters that the user is comfortable with, or that are visible on the specimen to be identified. Alternatively, the automated selection of optimal characters will guide the user through the identification process. The African key also provides possibilities to define a subset according to the geographical region. Datasheets provide information on general description, biology, distribution, management and host plant range. Hyperlinks to different sources on the internet provide additional information as well as libraries of virtual collections through high resolution images.

Conclusion: The developed keys are freely accessible and downloadable both for Apple and Android mobiles. It is anticipated that they will facilitate identification of targeted fruit flies for the non-specialist. It is also hoped that they will allow involvement of non-professional citizens in surveillance of pest species including potential invasive ones.

Keywords: taxonomy; species identification; market access; invasive pests

Poster: Genetics and Biotechnology

A phylogenomic approach to better understand the evolution of host-plant preferences in frugivorous *Dacus* (Tephritidae)

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The genus *Dacus* Fabricius is predominantly African where it is represented by 194 described species, including major pests such as *Dacus ciliatus*, *Dacus punctatifrons* and *Dacus vertebratus*. This genus is closely related to the genus *Zeugodacus* (formerly recognized as subgenus of *Bactrocera*)which includes the widely distributed cucurbit pest *Z. cucurbitae*. The available molecular phylogeny of *Dacus* do not corroborate the monophyly of a number of subgenera and species groups as described in previous taxonomical revisions and strongly suggest the need of further research on this group.

The members of *Dacus* seemingly have restricted host-plant associations, in particular with the families Cucurbitaceae, Passifloraceae and Apocynaceae. Previous phylogenetic analysis within this genus has shown a clear association with host plant range and the molecular phylogeny of African *Dacus* (at least for the species with known feeding preferences). Their monophyletic groups largely correspond to species feeding on either Apocynaceae or Passifloraceae, while a vast majority of cucurbit feeders was paraphyletic.

To investigate if the insect-host plant association patterns persist with a higher resolved phylogeny, we provide an extended phylogeny including >40 African *Dacus* species (as well as *Bactrocera* representatives) based on whole genome sequencing (WGS). Short read sequencing was done with Illumina at a coverage of x10. SNPs were collected after aligning all samples to a reference genome and performing variant calling on this alignment. After filtering the SNPs were used to construct a neighbour-joining tree. This study is part of a larger research on the evolution of host-plant preferences in frugivorous African tephritids.

Keywords: Host preferences, integrative taxonomy, phylogenomics

Poster: Genetics and Biotechnology

Bacterial symbiont diversity in and out of *Bactrocera* fruit flies in Okinawa, Japan

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Background: For over a century, fruit fly species in the genus *Bactrocera* have been reported as hosts of gut bacteria, with their beneficial roles gradually uncovered, such as enhancement of reproductive fitness and decomposition of plant tissues, toxins or even pesticides. However, nothing is known for gut bacteria of wild *Bactrocera* flies in Japan, including Ryukyu Islands where we face the highest risk of pest invasion and richest species diversity. In addition, relationships between gut bacteria and host plant utilization has been elusive in many tephritid species. Here, we investigated the diversity, phylogeny and localization of gut bacteria associated with tephritid flies in Okinawa, featuring both in (gut) and out (host plant) of flies for understanding the whole symbioses.
Methods: We collected over 300 wild fly specimens in total nine *Bactrocera* /*Zedodacus* species and 110 plant fruits in Okinawa, and extracted their DNA for 16S rDNA amplicon sequencing and community structure analyses. In parallel, digestive tracts of the flies were dissected, homogenized, diluted and plated on culture media for bacterial colony isolation. Complete 16S rDNA sequences were obtained for molecular phylogenetic analyses and operational taxonomy unit (OTU) classification. Fly tissues, eggs and plants were microscopically inspected by fluorescent *in situ* hybridization (FISH). We preliminarily tested antibiotics-based procedures to remove gut bacteria from *B. latifrons*.
Results: Our amplicon sequencing analyses revealed diverse bacterial genera in all fly species, but majority of those are restricted to two phylums, Proteobacteria and Firmicutes. OTU compositions did not reflect insect species nor host populations, but plant fruit types and origins. In *B. latifrons* populations, weighted PCoA showed similar distributions, but that of unweighted produced scattered plots by different islands and host plants, indicating minor OTUs might be affected by these factors. Comparisons between flies and host fruits detected common OTUs, suggesting that flies share gut symbionts within infested fruits. Cultivation and molecular characterization of >1,500 bacterial isolates from fly guts confirmed the similar bacterial diversity and some OTUs were host-plant specific. Laboratory-reared flies maintained the same OTUs over several generations. FISH observation revealed concentrated bacterial cells in midguts of later-stage larvae and adults, in addition to eggs and host plants.
Conclusion: Gut bacteria of Okinawan tephritid flies generally differ among insect species, and populations, but remain conserved between brothers and sisters derived from the shared host fruits. *B. latifrons* harbours some typical gut bacteria in larvae and adults, and maintains them for multiple generations, presumably transmitted horizontally in rotten fruits. Collectively, these results suggest that the host plant type, horizontal and vertical transmission shape the gut microbiota in *Bactrocera* flies.

Keywords: microbiota, gut symbiont, host plants, Ryukyu Islands, FISH

Poster: Genetics and Biotechnology

***Bactrocera dorsalis* in the Indian Ocean: a tale of two invasions**

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Background: *Bactrocera dorsalis* (Hendel) is among the most widespread tephritid species, including established populations on islands in the Indian Ocean. This region is of great phytosanitary concern as it connects Asia, Africa and Europe through economic, cultural and political ties. Although the invasion history may be estimated by dates of first recordings, a genomic approach has not been adopted yet. We aim to genetically analyse the islands' populations and reconstruct the invasion routes into and within the region. The obtained results may provide novel insights, useful for phytosanitary policymaking.

Methods: Genomic DNA sequence data were collected from 230 *B. dorsalis* specimens by PE Illumina Novaseq 6000 Whole-Genome Sequencing (12x coverage, read size 150bp). The resulting reads were mapped to a *B. dorsalis* reference genome followed by SNP calling and filtering. Invasion history was reconstructed by assessing genetic parameters such as genomic diversity and differentiation metrics. A population level maximum-likelihood tree reconstruction, principal component analysis, ancestry analysis, kinship networks, and runs of homozygosity (ROH) were performed to render a robust image of dispersal patterns within the study area.

Results: A western invasion pathway involved stepping-stone dispersal of *B. dorsalis* from the east African coast into the Comoros, Mayotte and Madagascar with a decreasing genetic diversity. The Mascarenes (Réunion and Mauritius) on the other hand were colonized directly from Asia and form a distinct cluster. The low nucleotide diversity suggests that only a few genotypes invaded the Mascarenes. The presence of many long ROH in the introduced populations are indicative for population bottlenecks, with a more severe bottleneck effect for populations along the western migration pathway than on the Mascarenes.

Conclusion: The western and eastern *B. dorsalis* populations in the Indian Ocean are genetically very distinct and likely originate from different sources. Our data shows that direction of dispersal of an invasive species might be more complex than initially assumed when looking at proximity between populations alone. We show that a genomics approach can assist in assessing the status of upcoming pest species and might help policymakers in improving phytosanitary protocols.

Keywords: phylogeography, dispersal, gene flow, whole-genome sequencing, population genetics

Poster: Genetics and Biotechnology

Comparative Genomic Hybridization (CGH) reveals sex chromosome molecular differentiation in *Anastrepha fraterculus* sp. 1 from Argentina

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Background: Anastrepha fraterculus, an economically important pest of fruit in several South American countries, is currently recognized as a complex of cryptic species composed by at least eight biological entities or morphotypes. This species has a diploid chromosome number of 2n = 12; a male heterogametic sex chromosome system (XX/XY) and five pairs of autosomes. In Argentina the presence of a single morphotype (*A. fraterculus* sp. 1) has been described, being the only one in which chromosomal polymorphisms have been reported so far. The polymorphisms involve the size, distribution and composition of the heterochromatin blocks on the sex chromosomes and allow defining two variants of the X chromosome (X_1 and X_2) and two variants of the Y chromosome (Y_5 and Y_6).

Methods: In this work, we use the comparative genomic hybridization (CGH) technique to evaluate the molecular differentiation between sex chromosomes and contribute to the knowledge related to its origin and evolution.

Results: Both X chromosomal variants presented the same signal patterns: positive hybridization with the female probe, excluding telomeres and including the distal satellite of the X_2 variant. In contrast, chromosome Y_5 showed a positive centromeric hybridization with the probe coming from the female genome and exclusively male DNA in the rest of the arms; evidencing a molecular differentiation in process. We confirm that *A. fraterculus* autosomes do not accumulate sex-specific differences because they showed a balanced signal.

Conclusion: These findings provide new knowledge that would reinforce the idea of a common origin of the X chromosome variants (X_1 and X_2), with a subsequent differentiation of the Y_5 chromosome, and provide evidence on the ongoing evolutionary process in *A. fraterculus* sp. 1. We will discuss our results considering previous studies in other insect species and the potential contribution of this novel information to the development and implementation of sustainable and specific control techniques against *A. fraterculus*.

Keywords: *Anastrepha fraterculus*, sex chromosomes, Comparative Genomic Hybridization, Sterile Insect Technique

Poster: Genetics and Biotechnology

Comparative larval microbiomics of cucurbit feeding fruit flies (Diptera: Tephritidae)

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Insect diversity appears to be closely linked with the evolution of phytophagy with most phytophagous insects showing a strong degree of specialization for specific host plants. However, many plants produce toxic secondary metabolites that protect them against phytophagy. So, how phytophagous insects can become specialized feeders while facing these secondary compounds remains to be clarified.

Experimental evidence suggests that microbial symbionts associated with insects might play a crucial role in facilitating insect host plant specialization. In this respect, true fruit flies (Diptera, Tephritidae) exhibit a degree of host plant specialization that ranges from monophagy to polyphagy. While evidence for a microbial role in host plant use has been provided for some monophagous frugivorous flies (Diptera, Tephritidae) such as *Bactrocera oleae*, further experimental data are needed from other species in this family. Among them, the oligophagous cucurbit feeders from the closely related genera *Zeugodacus* and *Dacus* are of particular interest due to their "intermediate" nature between generalism and specialism and their ability to feed on highly toxic plants. We explored the relation between tephritid fruit flies, their microbiomes and their feeding on various cucurbit host plants.

Third instar larvae of the cucurbit feeding tephritids (*Zeugodacus cucurbitae*, *Dacus ciliatus* and *D. bivitattus*) were recovered from three cucurbit host plants (*Citrullus lanatus*, *Cucumis sativus* and *Cucurbita maxima*) in Morogoro (Tanzania). A non-tephritid fly (*Atherigone orientalis*, Muscidae) that also feeds on cucurbits was included for comparison. Approximately 200 larvae were processed and individually subjected to microbiome profiling by 16S rRNA (V3-V4 region) metagenomics.

We explored the similarity of the larval microbiomes between different fruit fly species and the differences between larval microbiomes of larvae feeding on different cucurbit hosts. Metagenomic data were processed using the DADA2 pipeline. Differences in microbiome diversity between different fly species and between larvae from different cucurbit host plants were tested using ANOVA and PERMANOVA. Abundance-Ubiquity tests were used to verify the occurrence of core microbiomes (i.e. shared bacterial patterns) between species or between larvae feeding of the same host. Analyses are ongoing and results of these metagenomic comparisons will be presented and discussed.

Keywords: microbial symbionts, 16S rRNA metagenomics, phytophagy, plant-insect interactions

Poster: Genetics and Biotechnology

Cytogenetic analysis of the centromeric heterochromatin in mitotic metaphase chromosomes of two fruit fly species (Diptera: Tephritidae) in Bangladesh

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Background: *Bactrocera zonata* and *Zeugodacus tau* are major agricultural pests in tropical, sub-tropical and temperate regions of the world including Bangladesh. In this study, we described C-banded heterochromatin banding patterns in mitotic metaphase chromosomes of *B. zonata* and *Z. tau*. Distribution patterns as well as size and volume of C-banded heterochromatin blocks of these species are discussed.

Methods: Colonies of *B. zonata* and *Z. tau* maintained in the Fruit Fly Lab of Cytology and Biocontrol Research (CBR), IFRB were used to prepare C-banded mitotic metaphase chromosomes from neural ganglion of the third instar larvae of both species. Photographs were taken using a Zeiss AXIO Lab A1 microscope.

Results: Karyotypes of these species exhibit variation in size and shape of the sex chromosomes. X chromosome lengths of 3.09 µm and 3.50 µm were observed for female and male of *B. zonata*, respectively; vs. 5.48 µm and 5.55 µm for female and male of *Z. tau*, respectively. In *B.* zonata, pericentric C-bands with 18.33% heterochromatin are observed in the female X chromosome; in males, the X chromosome has heterochromatin (17.11%) in the centromere region. A faint C^+ band in the telomere region of the short arm is located in the X chromosome in both sexes. This band consists of 17.45% heterochromatin in females and 17.03% in males. The dot like Y chromosome is highly heterochromatic and in most cases full of c-heterochromatin. In some preparations, variation was also observed where heterochromatin was accumulated in partial regions of the Y chromosome. In the autosomes, C-bands are preferentially located in their pericentric region of the chromosomes. In *Z. tau*, the C-banding pattern reveals that the X chromosome possesses heterochromatin in one arm and the opposite arm is euchromatic. A prominent C-banded block (67% in females and 63% in males) covers the whole long arm of X chromosome in both sexes with a telomeric C^+ band (13.97% in female and 15.54% in male) in their short arm. In most cases, the Y chromosome is a small dot and highly heterochromatic. Heterochromatin accumulation was found as an elongation of the dot. Similarity was found with *B. zonata* for autosomes, i.e. C-bands are observed in pericentric region of the chromosomes and their distribution is also described.

Conclusion: Cytogenetic information obtained from this study could enhance understanding of the role of heterochromatin in cytotaxonomy and evolutionary facts of the cryptic species of dipteran insects. The results could open new possibilities for research on heterochromatin diversity at the cytogenetic level among different Tephritid fruit fly species.

Keywords: Tephritidae, heterochromatin block, sex chromosomes, C-bands

Poster: Genetics and Biotechnology

Effects of cucurbit diet, altitude and field management practices on the gut microbial patterns of the melon fly *Zeugodacus cucurbitae* (Diptera, Tephritidae)

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The gut microbiome of frugivorous tephritid pests has been shown to have an impact on a wide range of fitness traits such as survival, egg production and maturation, physiological development, foraging behaviour, oviposition site selection and parasite resistance. Describing factors and processes affecting microbiome diversity can thus be of great importance for a better understanding of the ecology and management of these pests.

Our research focusses on the combined effects of crop host, altitude/climatological conditions and farming method on the gut microbiome of the melon fly *Zeugodacus cucurbitae* in small scale farms in Tanzania. The two farming methods we compare are conventional (with the use of pesticides and herbicides) and agroecological (without chemical control methods). A balanced experimental design was implemented on 12 small farms, half of them located in the lower plains at ~500m in altitude and half located in the Uluguru mountains at an altitude of ~1000m. All farms grew two host crops (watermelon, *Citrullus lanatus* and cucumber, *Cucumis sativus*).

Larvae were collected during eight days and immediately stored in 100% ethanol. DNA of the whole larvae was extracted and for each sample the larvae was identified through COI DNA barcoding. For each of the 12 plots, four *Z. cucurbitae* larvae were subjected to microbial profiling via metagenomic amplification of the V3-V4 16S rDNA. Amplicon sequencing data were analysed using DADA2 and subjected to a permutational analysis of variance (PERMANOVA) to include farming method, altitude and crop host as main factors.
The analysis of data is currently in progress and will help disentangling the relative contribution provided by different factors to the gut microbial diversity of *Z. cucurbitae*.

Keywords: Melon fly, microbiome diversity, alternative farming practices

Poster: Genetics and Biotechnology

Genome-wide analysis of sympatric populations of two hybridisable bactroceran species identifies diagnostic genetic markers on putative X-linked genomic scaffolds

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Background: Despite the lack of post-zygotic reproductive barrier, *Bactrocera tryoni* and *B. neohumeralis* remain separate species that share an extensive sympatric range from northern Queensland to northern New South Wales. The quest for natural hybrids initially focused on the recovery of phenotypic (callus colour) intermediates, followed by microsatellite and nuclear gene markers, but have so far produced inconclusive results. Furthermore, diagnostic markers differentiating the three members of the tryoni species complex (*B. tryoni*, *B. aquilonis* and *B. neohumeralis*) have not been found despite intensive research efforts.

Methods: A genome-wide analysis was conducted using ~25,000 DArTseq markers on 604 flies (359 *B. tryoni* and 245 *B. neohumeralis*) from 52 populations collected across Australia and Torres Strait Islands, including ten sympatric populations (Brisbane, Cairns, Cape Tribulation, Cape York, Coen, Cooktown, Mapoon, Mareeba, Rockhampton and Weipa), to evaluate population structure and gene flow between these two species.

Results: Clustering analyses of ~25,000 markers showed that across the ten sympatric populations, two distinct clusters were formed representing members of each species, and detailed interrogation of genetic polymorphisms identified one putative natural hybrid from Mareeba. Furthermore, considering the *B. neohumeralis* individuals from 12 Torres Strait Island populations and *B. tryoni* individuals from five southern populations (Victoria and New South Wales) as the two parental genotypes, we isolated 35 markers that show the highest differentiation between the two species. Based on the new *B. tryoni* reference genome, ten of these 35 markers appeared to be single copy loci, scattered across multiple genomic scaffolds that were not assigned to any autosomes. Most of these putative diagnostic markers are found in a 23 Mb scaffold, which is believed to be a segment of the X-chromosome in *B. tryoni* based on strong *Bactrocera-Drosophila* synteny evidence.

Conclusion: Our genome-wide survey of *B. tryoni* and *B. neohumeralis* populations confirmed the weak gene flow between the two taxa despite sympatry and uncovered putative X-linked markers that could be developed into routine species diagnostic assays.

Keywords: Genome-wide markers, diagnostic markers, hybridisation, ILS

Poster: Genetics and Biotechnology

Genomic characterisation of the population structure of *Ceratitis rosa* and *C. quilicii* (Diptera, Tephritidae) in southern Africa

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Background: Ceratitis rosa Karsch and *C. quilicii* De Meyer, Mwatawala & Virgilio are two economically important frugivorous pests with partially overlapping ecological requirements and distributions throughout South and East Africa. Due to the recent split of C. *rosa* into *C. rosa* (Natal fruit fly) and *C. quilicii* (Cape fruit fly), the distributions of these pests are not exhaustively known and there is an urgent need for additional data to fill the current knowledge gap. A recent approach promoted, *inter alia*, by researchers of the Stellenbosch University allows integrating existing population genetic and distributional data with estimates of landscape connectivity (climate, vegetation, and topography) to generate predictions of the most likely dispersal pathways. In this framework we are now providing a first description of the population genomic structuring of these two species in southern Africa. Metrics obtained from levels of population structuring and connectivity across populations will be used in the framework of the project DISPEST, funded by the Belgian Development Cooperation.

Methods: More than 300 specimens were sampled across South Africa and Mozambique from 20 sympatric and allopatric populations of *C. rosa* and *C. quilicii*. Following DNA extraction and quality check, specimens were subjected to Whole Genome Sequencing (genome coverage10x) on Illumina Novaseq 6000. The resulting paired end reads (150bp, 6Gb / specimen) were aligned to a reference genome of *C. capitata* before SNP calling and filtering. Data analysis is currently ongoing and will allow inferring admixture across samples (as calculated from ancestry coefficients), and general population structuring as inferred from tree reconstructions and analysis of principal components (PCA).

Results and Conclusion: The project DISPEST is providing tools to evaluate the risks that *C. rosa* and *C. quilicii* pose to different crops and horticultural industries in southern Africa. In this respect, defining their putative dispersal patterns as function of landscape barriers and population structure and connectivity will help defining the spatial scale at which to apply suitable control strategies.

Keywords: genetic structuring, gene flow, connectivity

Poster: Genetics and Biotechnology

Insights on the genetic variability of long-term *Anastrepha fraterculus* colonies

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Background: Microsatellite markers has been successfully used for the assessment of the intra-specific genetic diversity in wild and laboratory populations of *Anastrepha fraterculus* (Wied.) of the Brazilian-1 morphotype in Argentina since 2014. However, the variations of genetic variability driven by the extended mass-rearing of many laboratory strains of this pest remain unknown. DNA samples from two *A. fraterculus* strains that were kept under semi-mass rearing conditions at CENA/USP for two years were examined in an effort to shed light on this matter.

Methods: The two laboratory strains (*Piracicaba* (PIRA) and *Vacaria* (VAC) strains) of the Brazilian-1 morphotype had been reared for 121 and 42 generations, respectively. Adult flies were randomly sampled from 6 generations of both colonies (*i.e.*, generations F_{107} to F_{110}, F_{114} and F_{121} of PIRA; and F_{23}, F_{27}, F_{31}, F_{35}, F_{39} and F_{42} of VAC), fixed in ethanol and kept at -80°C. Total DNA extractions were performed using the CTAB method. Microsatellite markers included *loci* AfA10, AfA112, AfA120, AfA122, AfC103, AfD4, AfD12 and AfD105. PCR amplifications were carried out under same conditions with the exception of the AfA112 locus. Labeled microsatellite fragments were multiplexed according to the fluorescent dye incorporated in the forward primers. Allele scoring was conducted in an ABI3130XL automatic sequencer. Indices of genetic diversity *per* population, total number of non-redundant alleles and unique alleles were obtained using '*basicStats*', '*divBasic*', and '*allele.dist*' functions implemented in the statistical environment R.

Results: Fragment data files were analysed (n=1,152 FSA profiles analysed in total). Clustering analysis using DAPC indicated low level of genetic structure among generations in both strains. However, a slight differentiation was detected in individuals sampled from generations 109 to 114 of PIRA, which experienced a "bottleneck effect" at F_{107}. The effects on the genetic diversity could be easily identified by the high density of individuals carrying a small number of different alleles at F_{108}, what resulted in a deviation from the Hardy-Weinberg equilibrium on F_{109}. Interesting, the F_{110} experienced a gain of genetic diversity (uHe from 0.45 to 0.54) and the appearance of two rare alleles, which were lost at subsequent generations.

Conclusion: These findings show how even little variations in population size have the potential to cause significant impacts on the genetic diversity of inbred strains. The results also suggest that rare alleles tend to disappear quickly in long-term breeding strains, in which genetic diversity dynamics favour homogeneity. In fact, as a result of genetic diversity loss over the longest domestication time, PIRA appears to be more homogeneous than VAC.

Keywords: Fruit Flies; Population Genetics; Microsatellites.

Poster: Genetics and Biotechnology

Linking genomes to Museum vouchers:
an open-access database for African "true" fruit flies (Diptera, Tephritidae)

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Background: Museum specimens are of particular importance for research on taxonomy, systematics and biogeography and provide tools to tackle a wide range of scientific questions in disciplines such as ecology, evolution and conservation. The rapid advances of -omic technologies have led to a substantial reduction in costs, so that the routine whole genome sequencing (WGS) of museum specimens represents a cutting-edge intervention to valorise the collections' genetic resources. In this context, the Joint Experimental Molecular Unit (JEMU) of RMCA-RBINS is defining the best practices for the genotyping of suboptimal insects. This approach relies on the archiving of *genomic vouchers* as a complement to the archiving of morphological and digital museum vouchers.

Methods: The performances of (a) different DNA extraction procedures and kits and (b) different genomic library preparation approaches were quantitatively compared on approximately 300 tephritid vouchers of different ages sampled from the collections of RMCA. After DNA extraction and quality check, samples were subjected to WGS at 10x coverage on an Illumina NovaSeq platform (150 PE reads, 6Gb raw data output / sample). Relationships between quantity/quality of extracted insect DNA (as estimated by fluorometric methods and by levels of DNA fragmentation) and quantity/quality of WGS output (including total amount of filtered reads, proportion of high quality reads, etc.) were investigated using a correlative approach.

Results and Conclusions: Correlation was observed between sample age, DNA fragmentation and WGS performances. Accordingly, a cost- and time-effective approach to DNA extraction and genomic library preparation has been defined for insect vouchers collected up to 30 years ago. A decision map on wet- and dry-lab procedures for the WGS of museum insects is proposed to optimize results based on initial quality checks. These results show that *a priori* and large-scale genotyping of relatively fresh collection specimens represent a complementary and cost -effective approach to the targeted genotyping of degraded museum specimens. The WGS data and metadata obtained for more than 300 fruit fly vouchers have been archived on the RMCA servers. The setup of an open-access database for voucher archiving (including voucher metadata, genomic data, digital imaging and linked to the Global Genome Biodiversity Network) has been initiated and will be finalized by 2023 in the framework of the projects InsectMOoD and SYNTHESYS+ (respectively funded by the Belgian Science Policy and by the European Union).

Keywords: Museomics, genomics, genomic voucher, DNA archiving

Poster: Genetics and Biotechnology

Melon fly research in Thailand: meeting our greatest export challenges

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Background: The melon fly, *Zeugodacus cucurbitae* (Coquillett), causes great economic losses in fruit and vegetable crops. It is a quarantine pest species for many countries which import host commodities from Thailand or are potential new markets for Thai exports. Larvae of melon flies are intercepted by quarantine inspections, but their morphological similarity to other fruit fly species makes identification difficult. Rapid, accurate identification of immature fruit flies associated with imported or exported fresh produce is essential to ensure appropriate biosecurity decisions at quarantine barriers or where commodities are inspected prior to export.

Methods: Species-specific primers and a lop-mediated isothermal amplification (LAMP) primer were designed by amplifying the cytochrome oxidase I (*cox1*) gene to differentiate *Z. cucurbitae* for all life stages.

Results: The species-specific assay and the LAMP primers demonstrated high specificity, sensitivity, and reliability for *Z. cucurbitae* when validated against representatives of 20 fruit fly species. This study demonstrated the feasibility of using species-specific diagnostic tools and LAMP primers for identifying all life stages of melon flies from populations throughout Thailand. Both tools were evaluated on samples intercepted by plant inspections at Suvarnabhumi airport of agricultural products destined for export from Thailand.

Conclusion: The species-specific assay and LAMP primers are suitable tools for reliable, simple, low-cost and rapid on-site identification of *Z. cucurbitae*, to facilitate accelerated export control processes for plant commodities. This tool will also be useful for domestic quarantine, monitoring and control of *Z. cucurbitae*. The present study is a model for developing diagnostic techniques for various pests which will in turn: promote trading partner confidence in Thai certification systems and enhance the diversity, quality and value of Thai agricultural products.

Keywords: Melon fly, *Zeugodacus cucurbitae*, fruit flies, Species-specific primers, LAMP

Poster: Genetics and Biotechnology

Optimal amount of yeast in diet to produce larvae of *Anastrepha ludens* (Diptera: Tephiritidae) as a source of protein

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Background: Entomophagy represents a promising resource in human nutrition for its development, as a viable and sustainable alternative source due to its richness in proteins.
To this end, fruit fly larvae of the Tephritidae family have been used as a protein alternative, which represents an alternative protein due to advances in their production under mass-rearing conditions and their safety. However, among the components of the larval diet currently used, yeast as one of the protein sources is one of the most expensive.
The reason for which it is sought that the amount of yeast in the larval diet can be modified to reduce its costs, without affecting the larval yields (biomass), and that the diets contain the nutrients and adequate quantities, which are assimilable for their development.
Methods: 5 treatments were evaluated, with different concentrations of yeast, using the official formulation of the Moscafrut Program as a control. The treatments were 100%, 75%, 50% and 25%. At each treatment, the diet and larvae with different days of development were analyzed: transformation from egg to larva, larval weight, biomass, yield, survival, moisture, ashes, crude protein, fat and crude fiber.
Results: The transformation of egg-larva presented a higher percentage of transformation in the treatment of 75%, without significant differences being observed between the treatments of 100% and 50%, being 25% that presented the lowest percentage of transformation.
The larval weight was significantly reduced as the percentage of yeast in the formulation decreased. No significant differences in larval performance were obtained in diets prepared with 100%, 75% and 50% yeast.
The biomass decreased as the yeast concentration decreased, the differences between the 4 treatments being significant.
No significant differences were observed in the percentage of survival between the concentrations of 100%, 75% and 50%, being the 25% concentration of yeast that showed the least survival.
Conclusion: The analysis of the biological parameters evaluated showed positive results in terms of larval performance. However, reducing the amount of yeast results in longer larval development times.

Keywords: protein, larval diets, fruit fly

Poster: Genetics and Biotechnology

Repurposing molecular markers to detect field polyandry and quantify stored sperm in the Queensland fruit fly

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Background: Since the late 1990s molecular markers have been developed to investigate population structure and chromosome evolution in the Queensland fruit fly (*Bactrocera tryoni*; Qfly). In some regions of Australia, Qfly outbreaks are managed by Sterile Insect Technique, for which effectiveness is heavily influenced by sexual processes. Here we present our recent success in repurposing molecular markers to detect natural polyandry and to quantify stored sperm in mated females.

Methods: Ovipositing females collected in New South Wales (NSW) in early October 2019 and Queensland (QLD) in mid-December 2019 were genotyped for ten single-copy microsatellite markers dispersed across five autosomes in the Qfly genome. The presence of three or more alleles in any of these ten markers was taken as evidence that the female had remated. To determine the number of stored sperm in once-mated females, a quantitative real time PCR (qPCR) assay was developed based on a previously reported Y-linked sequence. Replicate sperm solutions of known quantity were used to prepare serial sperm dilutions to calibrate a standard curve, which served as the reference for estimating sperm number in each spermatheca of once-mated females from a domesticated line.

Results: The overall female remating rate was 54.2% (N = 48). Significantly higher remating rate was detected in the NSW collection (80.0%; N = 25) than the QLD collection (26.1%; N = 23). In our laboratory study, sperm number per spermatheca was on average 3,209 (N = 69). The maximum value for number of sperm stored was 10,199 and the peak value for absolute asymmetry between the two spermathecae was approximately half of this value.

Conclusion: We obtained the first evidence of polyandry in Qfly field populations by multi-locus genotyping of stored sperm from ovipositing females. Significant difference in the prevalence of remating was observed between the NSW and QLD collections, suggesting spatial and/or temporal variation. Our new sperm quantification method based on a Y-specific marker enables measurement of sperm number with increased accuracy, throughput and process flexibility, and provided a revised understanding of sperm storage patterns in Qfly.

Keywords: genetic markers, polyandry, sperm genotyping, microsatellites, *Bactrocera tryoni*

Poster: Genetics and Biotechnology

Targeting the white-pupae causal gene to develop a phenotypic trait for *Bactrocera tryoni* genetic sexing strain

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Background: Queensland fruit fly *Bactrocera tryoni* (Froggatt; Diptera: Tephritidae) is one of the worst invasive pests in Australia, causing physical damages to fruit and vegetables and thus, economic losses. Biological control is needed to control this pest, which requires the application of sterile insect technique (SIT). By using this technique, translocation-based genetic sexing strains (GSSs) of some fruit fly species such as *Bactrocera dorsalis*, *Bactrocera cucurbitae* and *Ceratitis capitata* have been generated in which males have wildtype brown pupae and the phenotype of females is white pupae. Therefore, it would be feasible to separate males from females for sterilization and release for pest control. In *B. tryoni*, there has been no GSSs applied for SIT, and thus, sterile males and females have been co-released into outbreak areas. Currently, the white-pupae causal gene, *Major Facilitator Superfamily* (*MFS*), has been identified in *B.tryoni* and targeted to produce a phenotypic trait for GSSs.

Methods: CRISPR-Cas9 mutagenesis was used to target the *MFS* gene in *B. tryoni*. Homozygous mutant strains were developed, followed by assessment of the phenotypes and fitness of the mutants.

Results: Two different *MFS* knockout mutant strains were generated by introducing frameshift mutations into the *B. tryoni MFS* gene using CRISPR-Cas9 mutagenesis. A range of pupal phenotypes (white, grey, white/grey striped), distinctive from the wildtype brown colour, was observed among the homozygous mutants of the same genotype. This mutant phenotype has been designated as “greyscale” in our laboratory to describe the spectrum of observed colours. *Greyscale* mutant pupae were separated from the mixture of wildtype brown and greyscale pupae using a mechanical seed sorter in a trial sorting experiment with an accuracy rate exceeding 90%. On-going fitness evaluations show that there is slight fitness cost due to this mutation.

Conclusion: Our study focused on the generation of a pupal phenotypic trait that can be used in the development of a *B. tryoni* GSS. Targeting of the white-pupae causal gene *MFS* in *B. tryoni* resulted in a range of white/grey pupal colours, which is different from the reported phenotypes in other tephritid species. Nonetheless, the pupal colour phenotypes observed in *B. tryoni* are distinctive from the normal brown pupal colour of wild flies and can be used as a phenotypic trait in developing a *B. tryoni* GSS.

Keywords: *Bactrocera tryoni*, biological control, *Major Facilitator Superfamily* gene, greyscale pupae, genetic sexing strain

Poster: Genetics and Biotechnology

The genetic basic for Tephritid *black pupae* phenotypes

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Background: Sterile insect technique (SIT) is an efficient and species-specific biocontrol strategy used to manage or eradicate pest populations. Insect genetic sexing strains (GSS) enable removal of females from factory reared flies to facilitate sterile male-only releases, and enhance the efficiency and cost-effectiveness of SIT programs. Visual sex-link pupal colour phenotypes can be used for mechanical separation of females and here we develop a potential GSS marker for the Australian horticulture pest Queensland fruit fly, *Bactrocera tryoni*.

Methods: CRISPR/Cas9 genome editing induced targeted knockouts of a *Bactrocera tryoni* melanin pathway gene, followed by assessment of the phenotype and performance of the knock-out strain.

Results: Targeted mutations in a *B. tryoni* melanin pathway gene resulted in black colouration of larval anal lobes, black puparium and a dark adult body colour phenotype. The dark phenotype of null mutants were remarkably similar to that of the *Anastrepha ludens black pupae* (*bp*) GSS, which is used for SIT pest control in Mexico. The *bp* locus is on the homologous *B tryoni* chromosome, suggesting the two phenotypes may share a conserved mechanism. Analysis of publicly available *A. ludens* genome data found no variation in *bp* or wild type DNA coding sequence of the melanin gene. Transcriptome datasets of wild type *A. ludens* and GSS brown pupae males expressed many genes known to play a role in melanin synthesis, while homozygous *bp* females lacked expression of the *B. tryoni* melanin pathway ortholog. Although high fitness costs of the *B. tryoni* black pupae strain was initially observed, repeated backcrossing and reselection generated a fecund and stable strain.

Conclusion: Functional knockouts of the *B. tryoni* melanin pathway gene produces distinctive black phenotypes which are similar to *bp* phenotypes reported in other species. We present strongly support conserved function between *B. tryoni* mutation and the putative *A. ludens bp* gene. This phenotype holds potential for developing GSS via a Y-chromosome translocation (Y;5). The melanic pupae phenotype of these mutants could be used as a selectable marker for the construction of GSS for SIT applications in other major insect pest species.

Keywords: *Bactrocera tryoni*, sterile insect technique, genetic sexing strain, black pupae, *Anastrepha ludens*

Poster: Genetics and Biotechnology

Transcriptome analysis of the effects of cuelure feeding on *Bactrocera tryoni* and their offspring

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Background: Feeding on cuelure has been shown to increase male mating success in *Bactrocera tryoni*, while males that have fed on cuelure sire male offspring with an increased foraging ability for cuelure. The increased male mating success in the parental generation may be due to an enhanced male pheromone and/or an enhanced male metabolism. The metabolism hypothesis has been inferred to be occurring based on male *B. tryoni* fed on zingerone, but has not been tested for flies fed cuelure. The reason for the lure response in offspring is entirely unknown. To understand the genetic mechanisms underlying these effects, we investigated the transcriptome responses of parental male *B. tryoni* fed on cuelure and their F1 male offspring.

Methods: Sexually mature parental *B. tryoni* males either fed or unfed on cuelure were released separately into cages with females to mate and generate F1 male flies. Sexually mature males from the F1 generation sired from cuelure males or control males and both parental treatment groups were sampled for transcriptomic analysis. Differential gene expression and gene ontology analyses were used to investigate changes in gene expression across treatments and generations.

Results: Our results demonstrate that 3100 genes were differentially expressed between the two generations. In the parental generation, 282 genes were differentially expressed between the cuelure fed and unfed flies. In the F1 generation, 102 genes were differentially expressed between the lure treatments. Thirty-nine genes were consistently differentially expressed among the lure treatments across generations; 28 were upregulated and 11 were downregulated in the cuelure fed flies. Hierarchical clustering identified three clusters, one of which contained six genes strongly upregulated in the cuelure conditions. Over-represented gene ontology terms in this cluster were largely associated with metabolic enhancement, including binding, catalytic activity, and response to stimulus.

Conclusion: Our results indicate that only a few genes are consistently upregulated in both male offspring sired from males fed on cuelure and the parental cuelure-fed generation. That few genes underlie the effects observed in cuelure-fed male *B. tryoni* and their offspring, plus the function of these genes, supports the metabolic enhancement hypothesis explaining the lure effects. The differentially expressed genes identified in this study make a sensible starting point for future research to better understand and manipulate the generational effects of lures observed in *B. tryoni*.

Keyword: cuelure, Queensland fruit fly, transcriptomics, differential gene expression

Poster: Genetics and Biotechnology

Two in one: phylogeographic analysis revealed multiple invasion pathways of *Bactrocera latifrons* into Okinawan Islands, southwest Japan

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Background: Invasive fruit fly, *Bactrocera latifrons* primarily utilizes Solanaceae fruit crops such as tomatoes and peppers in Okinawa, southwest islands of Japan. *B. latifrons* once invaded Yonaguni Is., Okinawa, and was eradicated in 2011 by the sterile insect technique. However, in 2010, just before the eradication, *B. latifrons* was found on Okinawa Is. and rapidly expanded its distribution throughout the whole Okinawa, resulting in re-invasion to Yonaguni Is. after 8 years. In addition to their invasion history into the two geographically distant (more than 600 km) islands, their distinct host preferences, where the former hardly used bird pepper but the latter mainly uses it, suggests that these two populations may be different strains derived from remote geographic origins. In this study, we phylogenetically estimated the distribution patterns of the putative two populations in Okinawa by sequencing multiple mitochondrial and nuclear loci, thereby extrapolating their invasion pathways.

Methods: We used live, dried or ethanol-fixed specimens (a total of 153 individuals) of *B. latifrons* captured from 15 islands between 2004 to 2020. We amplified and directly sequenced the COII (480 bp) and ND4 (720 bp) regions of the mitochondrial DNA along with two nuclear genes (0.7 kbp each). Phylogenetic analyses of the sequences were performed by maximum-likelihood estimation. For representative genotype specimens, we also determined COI (1.5 kbp) and complete mitochondrial genome sequences, and analyzed their detailed phylogenetic positions with the sequences collected in other areas (China, Malaysia and Hawaii).

Results: Our detailed phylogenetic analyses revealed two major clades. All specimens collected in Okinawa Prefecture excluding Yonaguni Is. were assigned to the major single clade. This clade is closely related to those from China, Malaysia, and Hawaii. This suggests that the strains found in Okinawa and Hawaii may have invaded from China or Southeast Asia. On the other hand, 29 out of 36 specimens collected on Yonaguni Is. formed a distinct independent clade in the tree, suggesting at least two separate invasions into Yonaguni Is.. Our comparative analysis of mitochondrial and nuclear genes also indicated interbreeding and introgression of the two strains in Yonaguni Is.. Additionally, the rate of the Yonaguni strain was decreasing as of late, suggesting that it may be gradually being replaced by the late Okinawa one.

Conclusion: The population of *B. latifrons* that first invaded Yonaguni Is. is phylogenetically different from the population now widespread in Okinawa including Yonaguni recently. Only Yonaguni Is. had the multiple genotypes. These results support the hypothesis that the two populations originate from two distinct strains through multiple invasion routes at different times.

Keywords: solanum fruit fly, phylogenetic analysis, Ryukyu islands

Poster: Genetics and Biotechnology

Use of highly resolving molecular tools to assess the seasonal population dynamics of *Ceratitis capitata*

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Background: The Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), is an important pest species of African origin and has been introduced at various locations outside the species' natural range. It has made its way into Europe in the 19th century and became well established throughout the Mediterranean Basin. It is rapidly expanding its range and has the ability to colonize a wide variety of environments. Incursions of *C. capitata* beyond the northern margin of its range are increasing while established populations are pushing the boundaries of the species' current distribution further north. However, knowledge on annual and seasonal population dynamics of *C. capitata* is lacking. For this reason, we assessed whether populations within the core of European *C. capitata* distribution and populations at the edge of the distribution (marginal areas and satellite occurrences) show different genetic signatures in terms of genetic structuring and genetic diversity on a temporal scale.

Methods: To address our objectives, flies were repeatedly collected at locations in core, marginal and satellite regions within the *C. capitata* distribution. DNA samples were acquired and submitted for Whole-Genome Resequencing, followed by SNP-calling. Genetic differentiation (F_{ST}) was calculated and spatio-temporal structuring was assessed using admixture, principal component and cluster analysis together with kinship estimation. In order to test for significant differences between seasons, a series of permutation tests were performed.

Results: *C. capitata* in Europe exhibits a low level of spatial genetic structuring and could be a typical example of range expansion with a rapid invasion of suitable habitat. Additionally, we bring forward evidence of significant annual variation in genetic composition in populations of *C. capitata* at multiple locations. The case study of satellite occurrences (Vienna, Austria) suggested that isolated allotments where fruits and vegetables are grown on a small scale, likely serves as a habitat of transient nature with independent year-to-year genetic variation.

Conclusion: Here, we provide evidence for annual variation in genetic composition at both the core and margin of the distribution of *C. capitata*. The occurrence of *C. capitata* in Vienna is likely associated with annual fruit import and populations there are assumed to be transient. Geographical structuring of *C. capitata* in Europe is weak which is likely the result of intense gene flow and a shared ancestry.

Keywords: temporal genetic variation, Mediterranean fruit fly, population genetics, WGS

Poster: Chemical Ecology and Attractants

Assessing the invasion and spread of the melon fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae) in Mozambique and its implications on horticultural crop production and market access

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Background: The melon fruit fly, *Zeugodacus cucurbitae*, Coquillett (Diptera: Tephritidae) is among the most important pests of cucurbit fruits worldwide. Originating from Asia, it was first detected in Mozambique in 2013 in Mocimboa da Praia and Palma districts in the Northern Province of Cabo Delgado. Due to its status of invasive species, *Z. cucurbitae* is considered a quarantine species. The present study was conducted to assess *Z. cucurbitae*'s distribution, and spread in the country and its implications on cucurbit crops production and market access.

Methods: Trapping transects were established since November 2019 to March 2022 at all provinces in the South and Central regions and in Cabo Delgado province in the Northern region, at high and mid risk areas (orchards, villages, town markets, sites along the highways and at borders with neighbouring countries). Traps baited with Cuelure were placed at each sampling site and inspected once per month. Trap catches were expressed as captured flies per trap per day (FTD).

Results: *Zeugodacus cucurbitae*, was detected in several sampling sites in the Northern (Cabo Delgado province) and Central (Tete, Manica and Zambezia provinces) regions, where it was not reported previously. The highest FTD was reported at Condezi (FTD=16.0 adults per trap per day) followed by Caling (FTD=5.2), both in Tete province. In other sampling sites the FTD varied from 0.1 to 2.2. There has been no detection of *Z. cucurbitae* at the trapping sites in the southern provinces of Inhambane, Gaza and Maputo. Since its first detection in 2013, *Z. cucurbitae* has been present and restricted in the Northern part of Cabo Delgado Province. This is the first time it is detected in other parts of the country. These captures reflect permanent establishment of the fly in the detection areas. This fact is an indication that *Z. cucurbitae* is spreading and extending its distribution range in Mozambique.

Conclusion: The occurrence of *Z. cucurbitae* in the country, further aggravating the widespread presence of the other invasive species *Bactrocera dorsalis*, will have severe impact on fruit and vegetables production, as well as serious restrictions on the access to export markets. The results from the present study serve as an early warning information to the decision-makers and the national phytosanitary authorities to take appropriated measures to mitigate its economic impact in the country, at the same time minimizing its spread into unaffected areas.

Keywords: fruit fly, *Zeugodacus cucurbitae*, detection, spread, pest status, fruit fly

Poster: Chemical Ecology and Attractants

Attraction of Oriental fruit fly to □-caryophyllene

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Background: Methyl eugenol (ME) is a well known highly potent attractant for males of the Oriental fruit fly, *Bactrocera dorsalis.* However, ME only traps male flies thus, limiting the possibility of controlling entire adult fly population including females. Hence, the availability of a male and female bisexual attractant for this highly destructive fruit pest is most desired. The sesquiterpene compound, β-caryophyllene (CP) has been recorded as a strong male attractant for the guava fruit fly, *B. correcta* and CP attraction was also reported for females of *B. correcta* and *B. dorsalis*. In this work, the attraction of male and female *B. dorsalis* to CP was determined. The effects of sexual maturity on male and female attraction to CP was also evaluated. The involvement of antenna and maxillary palp in fly response to CP was also investigated.

Methods: **Laboratory cage and wind tunnel assays in a semi-outdoor environment were conducted on virgin and mated male and females of *B. dorsalis*. Ablation of olfactory organs were conducted under clean environment in a laminar flow.**

Results: We discovered that, like previous *B. dorsalis* attraction to ME that is a function of sexual maturity, the attraction of males to CP is also concomitant with attainment of sexual maturity albeit at older age. Males are attracted by the scent of CP and would fly excitedly to land on the source of the CP before feeding on it. CP-fed males demonstrated significantly higher mating success over non-CP-fed males. Gravid females however, when attracted to CP, do not feed on it unlike those males. Cage and wind tunnel bioassays further demonstrated that the attraction of *B. dorsalis* to CP is of short range only. Interestingly, we also demonstrated through combinations of antennal and maxillary palpal ablation, that both organs are required for optimal fly response to CP.

Conclusions: The present results suggest that CP, as a sex pheromone precursor is detected by both the antenna and maxillary palp, but at short ranges only.

Key Words: *Bactrocera dorsali*s, β-caryophyllene, wind tunnel, antenna, maxillary palp

Poster: Chemical Ecology and Attractants

Gut associated microbial volatile organic compounds (mVOCs): Potential to attract mature Queensland fruit fly, *Bactrocera tryoni*

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Background: Bactrocera tryoni is an economically important pest for which monitoring and management depends on cuelure, a male-specific attractant. There remains a need for female-specific synthetic lures. Studies of plant-insect-microbe interactions suggested that attraction towards the host is not only driven by plant volatile compounds but also the microbial volatile compounds (mVOCs) from host plant/fruit-associated microbes. Insects, including *B. tryoni*, acquire some of these host microbes during feeding and so the bacteria present in *B. tryoni* guts might trigger attraction to the host via mVOCs. Symbiotic interactions between fruit flies and their associated gut bacteria have been well-studied in several tephritids, including *B. tryoni*. However, the composition of volatile chemicals in these gut bacterial emissions and their role as mediators of fruit fly behaviour remain little studied.

Methods: We identified culturable bacterial species from wild adult *B. tryoni* via biochemical and molecular techniques and evaluated their attraction potential in trap assays. With the most attractive eight isolates, we collected the emitted mVOCs from the headspace of liquid bacterial cultures and identified them by gas chromatography-mass spectroscopy (GC-MS). We then investigated the electrophysiological and behavioural attractiveness of EAD/EPD-active mVOCs to mature male and female *B. tryoni* using gas chromatography coupled electroantennogram/electropalpogram (GC-EAD/EPD) and Y-maze olfactometer respectively.

Results: Molecular 16S rRNA sequencing identified 24 bacterial strains from wild adult male *B. tryoni* and 21 bacterial strains from female flies. The most frequent bacterial strains obtained from both male and female guts belong to Enterobactereaceae (64.25%) followed by Bacillaceae (8.25%). In trap assay, the most attractive bacteria were *Bacillus anthracis*, *Enterobacter cloaceae*, *Pseudomonas aeruginosa*, *Bacillus* sp., *Pseudomonas fluorescens*, *Enterococcus faecalis*, *Citrobacter freundii* and *Proteus mirabilis*. Young male and female flies exhibited a significantly lower response than mature male and female flies. The attraction of mature flies to different bacterial strains varied significantly, and was similar for male and female flies. GCMS analyses of these bacterial headspace extracts identified mVOCs with wide range of chemical groups including pyrazines, ketones, alcohols, aldehydes and esters. Many compounds were common to all isolates with few being species-specific. GC-EAD/EPD studies identified 17 electrophysiologically active volatile compounds from the extracts across all tested bacterial strains. Mature male and female *B. tryoni* exhibited varying attraction to these compounds in Y-maze olfactometer bioassays. The most attractive volatile for both sexes was 1-(2-aminophenyl)-ethanone, followed by geranyl acetate for females and geraniol for males.

Conclusions: Our study advances understanding of fruit fly-bacteria chemical relationships and provides insights that can support development of bisex synthetic lures as fruit fly pest management tools.

Key Words: *Bactrocera tryoni*, Queensland fruit fly, Microbial volatile, Biocontrol, Pest management

Poster: Chemical Ecology and Attractants

Identification of new attractants and behavior-modifying odors for the Mediterranean fruit fly and Caribbean fruit fly

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Background: The Mediterranean fruit fly, or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the most economically important pests of fruit crops worldwide. The Caribbean fruit fly, or caribfly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) is a quarantine pest of citrus in Florida, and a pest of guava and other specialty fruits in the Americas. Currently, trimedlure is used to monitor male medfly populations, and protein-based baits and lures are used to monitor male and female populations of both species. Developing alternative attractants based on host volatiles will improve monitoring for both species, and female-targeted attractants will have the added benefit of being compatible with sterile male release programs.

Methods: To identify alternatives to the current protein-based and male-targeted attractants, a series of electroantennogram (EAG) and small-cage assays were used to screen host fruits and essential oils to identify novel attractants for sterile male medfly and fertile caribflies.

Results: In small-cage bioassays, essential oils from the leaves and fruits of foetid juniper, *Juniperus foetidissima* Willd., were attractive to male medflies. Hydrodistalltion revealed that α-thujone and β-thujone composed almost 40% of the distillates from both the leaves and fruits. In subsequent behavioural assays, both α-thujone and β-thujone were less attractive than a known strong attractant, tea tree oil (TTO), though both α-thujone and β-thujone were more attractive than TTO after 90 minutes. Similar bioassays also showed that essential oils from crimson beebalm, *Monarda didyma* L., and oregano, *Origanum vulgare* L., were equally attractive to male medflies compared to the TTO control. GC-EAD (gas chromatography coupled with electroantennography) work is underway to identify the attractive compounds from *M. didyma* and *O. vulgare* that may have potential use as synthetic lures for male medflies. Additional GC-EAD and behavioural work is being conducted to screen for odors that are antennally sensitive and attractive to caribfly females from the headspace of ripe and unripe common guava, *Psidium guajava* L, and other caribfly host fruits.

Conclusion: This is the first report on attraction of male medflies to the essential oils from *J. foetidissima*, *M. didyma*, *O. vulgare*, and α- and β-thujone. Further, to our knowledge, this is the first report to analyse the volatiles from ripe and unripe guava fruit for attraction of female caribflies. Developing alternative attractants for medfly and caribfly will benefit regulatory agencies in designing improved pest detection programs, especially programs that support sterile male release.

Keywords: Mediterranean fruit fly, Caribbean fruit fly, Attraction, Electrophysiology

Poster: Chemical Ecology and Attractants

Plant-based attractant, zingerone and its analogs reveal structural requirements for attraction of Jarvis's fruit fly

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Background: The flowers of the sweet calabash and the tar tree are known to attract *Bactrocera jarvisi* males. Zingerone, a phytochemical, is known to attract several *Bactrocera* species, including *B. jarvisi*. We investigated the chemistry of the flowers of these two plants for presence of zingerone and related phytochemical fruit fly attractants and elucidated the functional groups of zingerone involved in the attraction of *B. jarvisi*.

Methods: The flowers of *P. maliformis* and *S. australiensis* were analysed by solvent extraction and gas chromatography-mass spectrometry (GC-MS). Zingerone analogs with side-chain, hydroxy, and methoxy group modifications were synthesised and tested for attraction of *B. jarvisi* and other fruit flies in the field.

Results: The flowers of both plants contained zingerone. Although *P. maliformis* is an introduced South American plant that is now commonly planted in areas where *B. jarvisi* is common, *S. australiensis* is native to Australia and is naturally sympatric with *B. jarvisi* suggesting a possible co-evolutionary relationship between the plant and insect. Field trials showed that the attractiveness of zingerone has very limited tolerance to structural modification. The most attractive zingerone analogs were alkoxy derivatives, with isopropoxy being the most attractive, followed by ethoxy and trifluoromethoxy analogs. The phenolic esters tested were also attractive with the response typically decreasing as size of the ester increased.

Conclusion: The carbonyl group, methoxy group, and phenol of zingerone are key sites for the attraction of *B. jarvisi* and there is little scope for structural modifications that can be made to zingerone (e.g., to increase volatility) without compromising attraction. These findings are important for future work in developing and optimising male lures for dacine fruit flies.

Keywords: passion fruit flowers, native cashew, *Bactrocera jarvisi*

Poster: Chemical Ecology and Attractants

Response of *Anastrepha fraterculus* Wiedemann (Diptera: Tephritidae) to synthetic semiochemicals

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Background: Specific attractants are a powerful tool for pest management. The efficient synthesis of epianastrephin and anastrephin, present in the male sex and aggregation pheromones of several *Anastrepha* species, and a synthetic analog of epianastrephin with two methyl groups (dimethyl), opened a new possibility to develop a specific attractant within this genus.

Methods: The response of *A. fraterculus* males and females, of different age and matingstatus, to synthetic semiochemicals was evaluated through electroantennographic analysis (EAG) and field cage experiments. Sexually-immature flies, sexually-mature virgin females and sexually-mature mated flies were tested. For EAG analyses, epianastrephin was included as a control to compare the antennae depolarization following dimethyl exposure. For field cages experiments, epianastrephin was provided jointly with anastrephin in two proportions and dimethyl was evaluated alone. In each cage, two traps hung from the roof of the cage, one with one attractant and one without attractant. One hundred flies of a given sex, age and mating status were released inside the cage and left for 16 h. Number of flies captured in each trap was recorded. Control cages contained a trap with five confined live mature males (leks). For immature flies, an additional positive control cage was set up with torula yeast. A liner model was built to analyze EAG response with sex, semiochemical and physiological condition as fixed factors. Field-cage data were compared using a Wilcoxon test for paired samples.

Results: Epianastrephin and dimethyl were EAG+ for all the flies' conditions; with epianastrephin eliciting the highest response for both sexes of all fly physiological conditions and immature flies showing greater responsiveness than mature ones. Attractiveness in the field cage experiments was dependent of the sex and the physiological condition. Immature flies showed attractiveness only to leks; virgin females to leks, dimethyl and both epianastrephin formulations; mature-mated males to leks, dimethyl and one epianastrephin formulation; and mature-mated females only to leks.

Conclusion: Our bioassays showed that traps baited with synthetic semiochemicals are attractive to *A. fraterculu*s males and females being this attraction dependent on the status of the fly. Dimethyl showed a promising performance since it elicited the same response than epianastrephin. The attraction observed to the leks in all mating status and ages evaluated suggests that the blend released by the calling males may contain food and host cues which could be acting as a sensory traps. The addition of any of these compounds in the synthetic attractants may results in a greater attraction and deserves further evaluation. Dose response experiments will provide additional information to move a step forward and validate the results obtained in open field conditions.

Keywords: epianastrephin, anastrephin, dimethyl, physiological status, electroantennography, field cage experiments.

Poster: Chemical Ecology and Attractants

Response of *Bactrocera carambolae* (Diptera: Tephritidae) to several protein waste products as a food bait

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Background: *Bactrocera carambolae* (Drew & Hancock) is a polyphagous fruit fly, which is an important pest of carambola, guava and mango. Various physical, cultural, biological and chemical control methods have been evaluated for fruit fly control, but the use of a food bait, especially protein to attract adult flies provides the best control to date. Protein bait is currently imported into Indonesia and is expensive as a result. To reduce this reliance on imports, studies were carried out to assess the attractancy of protein bait made from cheap and readily available local sources of protein.

Methods*:* Several types of waste products were assessed, namely ciu (traditional liquor) waste, beer dregs, beer liquid waste and fermented sticky rice and compared against commercial protein bait as a positive control and clean water as a negative control. The raw waste products were processed by mixing them with enzymes and proteins and fermenting the mixtures at 50°C for 24 hours. A Y-tube olfactometer was used to determine the individual responses of 800 adult *B. carambolae* toward four protein-waste products, one commercially available protein-bait and clean water.

Results*: B. carambolae* adults were attracted to all protein-waste products tested and the commercially available protein-bait as compared to clean water vapor as a control. The protein products derived from beer dregs attracted the highest number of flies (71%), followed by commercial protein-bait (65%), protein products from fermented sticky rice (60%), ciu waste (53%) and beer liquid waste (42%).

Conclusion*:* Local waste products which are cheap and easily available have the potential to be used in the manufacture of local fruit fly protein bait. The use of food-based baits is highly compatible with an Integrated Pest Management system for fruit fly pests in Indonesia. Further studies should include *B. dorsalis.* Laboratory experiments should progress to field-based testing.

Keywords: Fruit Fly, Food bait, Protein source, Olfactometer

Poster: Chemical Ecology and Attractants

Response to lures by *Bactrocera dorsalis* and *Ceratitis capitata* depends on physiological condition and temperature

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Background: The responsiveness of tephritid species to lures can change depending on fly age and adult diet. Time of day also affects lure responsiveness, and it is likely that other abiotic variables, such as temperature and humidity, have a similar impact on fruit fly lure response, and thus trap efficiency.

Methods: We determined how interactions between weather and physiology affect the lure response of *Bactrocera dorsalis* (Hendel) and *Ceratitis capitata* (Wiedemann). We tested methyl eugenol for *B. dorsalis*, EGOlure for *C. capitata*, and BioLure for both species. For each species and lure combination, response by groups of flies (n=25) was determined in a semi-field setting in relation to time of day (06:30, 10:00, 13:30 and 17:00), temperature and relative humidity, sex, diet (protein-rich and protein-deprived) and age (2, 10 and 20 days old).

Results: Male *B. dorsalis* were highly responsive to methyl eugenol but two-day old, immature males responded less than mature flies. Male *C. capitata* responded strongly to EGOlure regardless of age. As anticipated, females did not respond to these male-specific lures. Sex also influenced the response of *C. capitata* to Biolure, with more males responding to this synthetic food-based lure. Young, sexually immature *C. capitata* flies responded less to Biolure baited traps than older flies. Surprisingly, diet did not affect lure response of *C. capitata* to either EGOlure or Biolure. However, *B. dorsalis* response to methyl eugenol was decreased in flies deprived of protein. In both species, lure response was highest during later periods of the day, correlating with warmer temperatures.

Conclusion: Response to different lures is influenced by the environment and the physiological state of flies. This is likely a result of physiological condition impacting the degree to which a lure is considered attractive. Differences in response to lures throughout the day are likely due to the effects of temperature on metabolic rate and capacity for flight, and daily behavioural rhythms in foraging and mating. These results emphasise the need for integration of fruit fly biology with environmental variables in detection and surveillance programmes.

Key Words: Trap response, attractants, motivational state, IPM

Poster: Chemical Ecology and Attractants

Sustainable IPM Strategies to control fruit fly and its impact on fruit export for smallholder farmers

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Background: The fruit fly is a significant threat to the major fruit and vegetable growing regions worldwide. The crop losses have been recorded at over 50% if no control measures are applied. Chemical pesticides have a negative impact on the environment and it has been shown that resistance to these chemicals gradually develops against various fruit fly species including *Bactrocera dorsalis* and *Bactrocera cucurbitae*. We have conducted a large-scale, area-wide application of biorational male and female attract and kill crop protection strategies in several Asian countries. These strategies have resulted in over 90% fruit fly control using an affordable and low-cost system.

Methods: The male and female attractant-based attract and kill (A&K) systems were employed to control the adult population of *B. dorsalis and B. cucurbitae.* At the same time, entomopathogenic fungi were applied near the root zone to control overwintering pupae in the soil. The application rate was 100 bait female bait stations containing hydrolysed protein plus 0.2% alpha-cypermethrin and 40 male A&K bait stations containing 30% methyl eugenol plus 0.5% Spinosad per hectare mango orchard. Three kg of entomopathogenic fungi formulation was applied near the root zone per hectare. The trial was conducted following a randomised block design. The male and female population data and crop loss data were collected at regular intervals.

Results: Over 95% fruit fly control was been achieved and the rate of damage was only 4-5% in trial areas. It was an opportunity for commercial farmers to harvest insecticide residue-free mango and cucurbit vegetables in Bangladesh, Nepal and Pakistan. In Bangladesh, local farmers exported the whole harvest at an increased premium price of 25% more than conventionally chemical-treated orchards. This represents a big breakthrough for Bangladeshi farmers as it allows them to export to the UK and EU countries without restriction. The successful strategy will be replicated in the coming season and around 50,000 hectares will be treated with this combined strategy.

Conclusion: It was demonstrated that the biorational attract and kill system is a real alternative to chemical treatments for subsistence farmers. We have observed the application of entomopathogenic fungi made a significant difference in the early stage fruit fly population in fruit orchards. The technology has been adopted in the mango growing region to combat *B. dorsalis and B. cucurbitae.* For commercial use, this technology system has been approved by the local agricultural authorities in Bangladesh and Nepal. More detailed observations will follow on the contribution of entomopathogenic fungi in future trials.

Keywords: Attract and kill, oriental fruit fly, cucurbit fruit fly, *B. dorsalis, B. cucurbitae*, entomopathogenic fungi.

Poster: Chemical Ecology and Attractants

The epicuticular chemoecology of the melon fruit fly, *Zeugodacus cucurbitae* (Diptera: Tephritidae)

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Background: The melon fly, *Zeugodacus cucurbitae* (Coquillett), is one of the main pests of Cucurbitaceae crops worldwide. For many insect pests, the pheromones involved in mate choice drive efficient behaviour-based control strategies. However *Z. cucurbitae* produces a complex set of cuticular hydrocarbons, for which chemoreceptive and behavioural responses of conspecifics are not known. We delved into the epicuticular chemical ecology of this species to fill this knowledge gap.

Methods: Epicuticular samples were collected by diving whole insects in hexane solvent. Samples of different ages and sexes were analysed through two-dimensional gas chromatography (GCxGC) coupled to mass spectrometric detection (MS) and multivariance factorial analysis. The chemosensory response to natural and synthetic compounds in both sexes was tested through a chopping coupling of the GC with a triple electroantennogram detector (EAD_3) and an electropalpogram detector (EPD). This innovative techniques ensure an extensive probing of the insect olfactory sensitivity (n=5 to 10 per condition). Finally, the orientation behaviour of unmated females toward a synthetic pheromonal blend was tested in a 6-way olfactometer.

Results: A consistent differentiation of the chemical profiles over time was observed; in young individuals the chemical profiles did not differ between sexes, while sex-specific differences were highlighted in mature flies. The antennae of both males and females were sensitive to three identified male components whereas the palps were sensitive to the female component. Ongoing behavioural tests suggest that a male synthetic blend, with similar compound ratio presented in the natural extract, attracts unmated naive females. Finally, the chemosensory responsiveness of other tephritid species was also investigated, concluding that *Z. cucurbitae* uses species-specific olfactory receptors to detect these compounds.

Conclusions: Compared with previous studies, we detailed the basic knowledge on the temporal profiles of epicuticular hydrocarbon production in *Z. cucurbitae*. We also sorted the compounds by the levels of olfactory response they evoke, designed a male synthetic blend and showed that it attracts unmated females. Notably, one compound, largely highlighted in the literature as the main pheromone component for this species, didn't elicit any sensory response which challenges its status as a pheromone. Overall, this study scientifically extends our understanding of the melon fly chemical ecology, and may have potential implications for developing behaviour-based control tools and new tephritid lures.

***Keywords*:** cuticular hydrocarbons; pheromones; GC×GC-MS; GC-EAD; GC-EPD; olfactometer; attractant

Poster: Chemical Ecology and Attractants

Transcriptomic analysis of odorant receptors in Tephritidae

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Background: Fruit flies of the Tephritidae family (Diptera) are among, the main pests of fruit and vegetable production worldwide. Some species are particularly problematic in subtropical areas, and global warming facilitates their invasive potential (e.g. recent detections of *Bactrocera dorsalis* in Italy and France). An effective method to monitor and control these pests, in combination with other methods, is the use of attractants. However, the attractiveness of these compounds varies depending on the species, and the use of some of the compounds is regulated because of their toxicity. In this context, we seek to extend the range of attractants that can be used for fruit fly control, using an innovative reverse chemical ecology approach targeting odorant receptors (OR), proteins at the core of olfactory detection.

Methods: To address this issue, we first sequenced via Illumina and *de novo* assembled the transcriptomes of olfactory organs (antennae and maxillary palps) from nine species of fruit flies present on Reunion Island, namely *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, *Bactrocera zonata*, *Dacus demmerezi*, *Dacus ciliatus*, *Ceratitis capitata*, *Ceratitis catoirii*, *Ceratitis quilicii* and *Neoceratitis cyanescens*. We then annotated the repertoires of OR transcripts for each species and performed phylogenetic analyses.

Results: The reference transcriptomes from species belong to the Dacini tribe contained around 47,000 sequences encoding for proteins whereas the transcriptomes from species belong to the Ceratitidini tribe contained around 65,000 sequences. According to the Benchmarking Universal Single-Copy Orthologs (BUSCO) evaluation, the nine transcriptomes revealed a good completeness (>93% of complete BUSCO genes found). Sixty to 70 candidate ORs, including the obligatory coreceptor Orco, could be identified, revealing that these repertoires are of comparable size among Diptera. With 70% of full-length OR sequences, we could complete many previously described incomplete sequences.

Conclusions: The established transcriptomes and OR repertoires represent a master resource for the scientific community. They also open the route for differential expression analyses according to sex and tissues that may pinpoint key ORs for further functional analyses and identification of new volatile attractants.

Keywords: Tephritidae species, Reverse chemical ecology, Transcriptomics, Odorant Receptors (ORs), Attractants

Poster: Risk Assessment, Quarantine and Post-Harvest

Challenges and insights on methyl bromide fumigation treatment against the Queensland Fruit Fly, *Bactrocera tryoni*

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Background: Methyl bromide (MB) fumigation treatments are an important phytosanitary measure to meet quarantine entry conditions that control the spread of Queensland Fruit Fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) within Australia and to overseas countries and markets. However, MB treatment efficacy against *B. tryoni* varies across fruit and vegetable commodities and fresh produce biological factors that mediate efficacy are not sufficiently understood.

Methods: Advanced imaging techniques, including computer tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) have been used to expend our understanding on how the differences in fresh produce morphology, anatomy, and physiology affect or mediate the efficacy of MB fumigation against *B. tryoni*. Mass spectrometry imaging (MSI) on fruit tissues pre- and post-MB fumigation has also been trialled to investigate whether metabolite composition influences the fumigation efficacy

Conclusion: According to commodity type and condition, multiple advanced imaging and analysis of fresh produce morphological, anatomical, and metabolic characteristics can improve understanding and inform optimisation of MB fumigation treatments against *B. tryoni.*

Keywords: Queensland fruit fly, *Bactrocera tryoni*, methyl bromide, Computer Tomography & Mass Spectrometry imaging

Poster: Risk Assessment, Quarantine and Post-Harvest

Conditional non-host status of Australian Hass avocados against Queensland fruit fly (*Bactrocera tryoni*)

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Background: Avocados (*Persea americana*) are considered a poor host of Tephritid fruit flies, including Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). Host status is an approved phytosanitary measure used to prevent the entry or establishment of a pest into an area. Host status recognises the natural resistant properties of fruit at a specified stage of development and can underpin the development of protocols to facilitate market access within and between countries.

Methods: Field trials were carried out under semi-natural conditions using the procedure of the International Plant Protection Convention ISPM 37 "Determination of host status of fruit to fruit flies (Tephritidae)". Field trials were conducted in four commercial avocado orchards in Queensland and New South Wales, Australia, at the end of the season, in late winter and spring (August/September). Mesh cage sleeves were secured around avocado fruit-bearing branches, enclosing 2-4 avocados (>500g) at each location. Additional control cage sleeves containing a known host fruit (banana or tomato) were present at each location. Gravid mated female fruit flies (n=33-50) were released into the sleeves and remained there for 48 hours. After 48 hours, the fruit were cut from each branch and transported to the laboratory and each replicate was held separately. The number of pupae and larvae resulting from each sample was counted and weighed and results used to calculate the potential infestation level.

Results: No pupae were reared from undamaged, hard mature avocados in any of the four trials. Pupae were recovered from all control cages, with a total of 2,474 pupae recorded from 12 control cages. In total, the potential infestation level based on pupae recovered in control fruit across all trials was 3,262 insects. This equates to 99.9082% efficacy with 95% confidence.

Conclusion: Host status testing conducted under semi-field conditions, that used no-choice testing with extremely high pest pressure, demonstrated that Australian grown Hass avocados are a conditional non-host to *B. tryoni*. Data generated in this research corroborates the findings of many other Tephritid researchers who have shown that commercial first grade, hard mature, Hass avocados are a conditional non-host of other fruit fly species, including: *Anastrepha stricta*, *A fraterculus*, *A. ludens*, *A. obliqua*, *A. serpentina* and *Ceratitis capitata.*

Keywords: *Persea americana*, Hass cultivar, fruit fly, host status, Tephritidae

Poster: Risk Assessment, Quarantine and Post-Harvest

Determination of host status of *Citrus sinensis* to *Zeugodacus tau* populations

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Background: The fruit fly pest *Zeugodacus tau* sensu stricto can infest 77 plant taxa from 23 families under natural field conditions. Cucurbitaceae is the most preferred host family with 37 out of 77 taxa reported as natural hosts. Conversely, the host status of several species belonging to the family Rutaceae, such as *Citrus sinensis*, remains undetermined. Citrus species are known to be attacked by several polyphagous tephritid fruit flies. Hence, the determination of the host status of *C. sinensis* to *Z. tau* is urgently needed to support pest risk analysis.

Methods: The potential for wild populations of *Z. tau* from Baipayl, Fujian, and Palampur to infest *C. sinensis* was evaluated in non-choice tests using standard field cages under semi-natural conditions. For each *Z. tau* population, 100 mated couples were released into field cages and exposed for 24 h to four zucchini (control) or four *C. sinensis* (intact or perforated orange). Potentially infested fruit remained untouched to allow the insects to complete their development. The total number of dead larvae, live larvae, and puparia were recorded for each commodity. Furthermore, the percentage of infested hosts, the number of larvae per host, and the percentage of non-deformed adults emerged were calculated.

Results: *Zeugodacus tau* females showed a higher propensity to infest zucchinis, followed by perforated oranges and intact oranges. Females from Baipayl infested intact orange more often than females from other populations and together with Fujian-Wild females, exhibited higher propensity to infest perforated oranges compared to Palampur. Infested zucchinis yielded the highest numbers of larvae, followed by perforated oranges and intact oranges. Zucchinis infested by Fujian-Wild yielded the highest number of larvae among all hosts and populations, larvae infesting either intact or perforated oranges were found in higher numbers for Baipayl compared to Fujian-Wild and Palampur. No difference in adult emergence was found among *Z. tau* populations reared on zucchinis, but insects reared on oranges showed high variability and a trend for reduced emergence.

Conclusions: *Citrus sinensis* should be considered a conditional host to *Z. tau* because it can not only be infested by different *Z. tau* populations but also sustain their development to viable adults under semi-natural conditions.

Keywords: host status, *Zeugodacus tau*, *Citrus sinensis*

Poster: Risk Assessment, Quarantine and Post-Harvest

Disinfestation of 'Palmer' cv. mango with gamma radiation

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Background: Ionizing radiation can be used as a quarantine post-harvest treatment for several fresh fruit commodities, and phytosanitary irradiation treatment doses for certain mango cultivars, such as 'Tommy Atkins', 'Kent', and 'Ataulfo', have already been evaluated. Currently, the 'Palmer' cv. mango production has expanded in Brazil, aiming to meet a growing demand from the domestic and foreign markets. However, there are no studies to date on the effects of radiation on the quality of the fruits of this cultivar and if the generic dose of 150 Gy would be effective against the immature stages of different fruit fly species inside its fruits. This study, therefore, aimed to verify the efficiency of two generic doses of gamma radiation for disinfestation of ripe 'Palmer' cv. fruits infested with eggs of two fruit flies, *Ceratitis capitata* and *Anastrepha fraterculus* (Wied.).
Methods: To carry out this study, 48 pre-selected mangoes were used and sanitized and then divided into two groups of 24 fruits to be infested either by *C. capitata* or *A. fraterculus*. The infestation cages (50x50x50 cm) were set with ca. 4,000 medflies or 2,000 *A. fraterculus* flies, containing water and food *ad libitum*. After the adults reached sexual maturity, the mangoes were exposed, with only one fruit being placed per cage every 24 h. After the replacement, the fruits were irradiated within the next 24 to 48 h with 150 or 300 Gy of gamma rays at the panoramic *Gammabeam*-650® irradiator. For each treatment, including the control group, there were 8 replicates. After the irradiation, the containers with the fruits and vermiculite were placed on shelves for 9-14 days. The counting of larvae was performed daily from the moment when they started crawling out of the fruits and ceased with complete mango rot in control groups. Then, all the fruits were dissected in order to count the larvae that remained inside them. The means of the total numbers of larvae from each treatment were compared by the Tukey's test (α=5%).
Results: The mean number of third instar larvae obtained from the control fruits infested by *C. capitata* or *A. fraterculus* were 270±13 and 367±20.9 larvae, respectively. In contrast, no larvae were found in the irradiated fruits.
Conclusion: The generic dose of 150 Gy is sufficient and effective to disinfest 'Palmer' cv. mango fruits containing eggs or 1st instar larvae of *C. capitata* and *A. fraterculus*. Furthermore, the evaluation of the effects of different dose ranges on the physicochemical and organoleptic qualities of the fruits is being carried out.
Keywords: Phytosanitary irradiation, Medfly, South American fruit fly.

Poster: Risk Assessment, Quarantine and Post-Harvest

Distribution of *Bactrocera dorsalis* (Hendel) in Papua New Guinea: has this economic pest species extended its range to East New Britain Province of Papua New Guinea?

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Background: The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a serious pest of economically important horticultural commodities with major biosecurity and market access consequences. It is native to tropical Asia and was first reported in mainland Papua New Guinea (PNG) in 1992. Detailed trapping survey undertaken from 1997-2002 for whole of PNG confirmed its establishment in mainland PNG but was not recorded in the island provinces. PNG does not have internal quarantine restriction for movement of fruit fly host fruits from mainland to the island provinces, so we predicted *B. dorsalis* to have extended its range to the island province of East New Britain. This study aimed to determine 1) if *B. dorsalis* has extended its range into this island province, and 2) seasonal abundance of other economic pest fruit fly species in this province.

Methods: Pairs of fruit fly traps baited with cue-lure and methyl eugenol attractants were established in three strategically selected sites including Kokopo, Rabaul and Kerevat within the Gazelle district of East New Britain province from 2014-2019. These sites are regarded as the potential early detection sites of *B. dorsalis* into East New Britain province. Snapshot trapping survey was also undertaken in western parts of New Britain island. Flies caught in each trap were collected monthly and identified.

Results: Bactrocera dorsalis was not detected in any of the traps established in the Kokopo, Rabaul and Kerevat as well as from traps used for the snapshot survey. Apart from the twelve pest fruit fly species previously recorded, there were no new pest species recorded in East new Britain province. The abundance of three main economic species (*Bactrocera frauenfeldi*, *Zeagodacus cucubitae*, *Bactrocera musae*) was low in the first three years but increased in the latter three years of trapping, where abundance of each species differed between trapping sites.
Conclusion: The non-establishment of *B. dorsalis* in the island province of East New Britain was surprising given the invasive behaviour of *B. dorsalis* recorded in other countries, as well as the cultural obligations of Papua New Guineans to bring food items (e.g., host fruits) when travelling between provinces to visit relatives. A biosecurity risk pathway analysis could identify other non-obvious pathways which would be helpful for pest risk analysis planning of countries where *B. dorsalis* is not currently present (e.g., Australia). The difference in abundance of three other pest species in the last three years of the study was most probably affected by the specific habitat attributes within each trapping site.

Key Words: *Bactrocera dorsalis*, seasonal abundance, East New Britain province

Poster: Risk Assessment, Quarantine and Post-Harvest

Efficacy of low-dose methyl bromide against Queensland fruit fly (*Bactrocera tryoni*) in Strawberries for market access

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Background: Australian strawberries (*Fragaria x ananassa*) are hosts of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) and when grown outside of recognised Pest Free Areas must undergo a disinfestation treatment before being exported to countries with quarantine restrictions against this pest. Methyl bromide (MB) remains the predominant fumigant for phytosanitary purposes for disinfestation of fruits and vegetables, with quarantine applications exempt from the Montreal Protocol phase-out. Low-dose methyl bromide (LDMB) uses lower concentrations of methyl bromide than traditional schedules but applied for longer treatment durations. This is in line with International Plant Protection Convention (IPPC) recommendations that reduction of methyl bromide emissions can be achieved using reduced dosages where technically feasible. Low-dose methyl bromide treatments are currently used for exports of nectarines and peaches from Australia to China. This project aimed to generate mortality data on all immature life stages of *B. tryoni* with LDMB in strawberries.

Methods: Strawberries were infested with eggs of *B. tryoni* by placing them in rows into cages of mature adults for 5 minutes. One fruit per row was randomly allocated as a control and used to estimate the number of insects subsequently treated. The fruit were held at constant temperature until the insects had developed to each of the four immature life stages: mature eggs, first, second and third instar larvae.

Infested fruit were fumigated with MB at a nominal treatment dose of 18 g/m^3 at 18°C for 5.5 hours inside a 1 m^3 fumigation chamber positioned inside a refrigerated shipping container to maintain temperature. Gas chromatography was used to monitor the headspace concentration of MB during fumigation and the chamber air and fruit core temperature were recorded for the duration of each treatment.

Results: A minimum of three large scale trials were conducted against each life stage of *B. tryoni*. An estimated 61,437 eggs, 35,797 first instar larvae, 34,728 second instar larvae and 32,874 third instar larvae were treated with no survivors recovered. The lower mortality 95% confidence limit for each life stage was 99.9951%, 99.9916%, 99.9914% and 99.9909% true mortality respectively, which exceeds the requirements of most quarantine authorities of 99.99%.

Conclusion: The data shows that a treatment schedule of 18 g/m^3 MB applied at 18°C for 5.5 hours was efficacious against all life stages of *B. tryoni* in strawberries and will support development of international market access protocols.

Keywords: Tephritidae, Diptera, *Fragaria x ananassa*, market access, disinfestation, phytosanitary treatment

Poster: Risk Assessment, Quarantine and Post-Harvest

High tolerance of *Zeugodacus tau* third instar larvae to phytosanitary cold treatment

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Background: The pumpkin fruit fly, *Zeugodacus tau*, is a species of economic importance widely distributed in Asia with potential to infest over 75 fruit hosts. Phytosanitary treatment options available for *Z. tau* are limited to two phytosanitary irradiation doses (i.e., 85 Gy and 150 Gy) and two cold treatment schedules for guavas and citrus. The cold treatment of ≤ 1 °C for 17 days is the only stand-alone cold treatment schedule currently available for *Z. tau* in guava. Cold treatment options for *Z. tau* in citrus can only be used as part of a systems approach because efficacy has not yet been determined for treatments of 18, 20, and 22 days at ≤ 0.56, 1.11, and 1.67 °C, respectively. The objective of this study was to determine whether a treatment of ≤ 1.70 °C for 22 days can be used as a stand-alone measure against *Z. tau* in citrus.

Methods: Experiments were conducted using navel oranges naturally infested by *Z. tau* from Fujian, China. Most tolerant stage testing indicated the third instar as the least cold susceptible life stage of *Z. tau*. Hence, confirmatory tests were carried out exposing more than 30,000 third instar larvae to ≤ 1.70 ºC for 22 days. Treatment efficacy was determined by larval mortality.

Results: A total of 36,512 third instar larvae were exposed to ≤ 1.70 °C for 22 days. Four apparent survivors were observed during the post treatment evaluation. Of those four survivors, two were found as puparia inside the containers with the treated oranges and the other two were found as crawling and live colour larvae inside the commodity. The live colour larva was recorded as survivor because it was able to pupariate during the holding period after fruit dissection. Nevertheless, none of the four survivors developed to the adult stage.

Conclusion: The cold treatment of 22 days at ≤ 1.70 °C evaluated in our study prevented *Z. tau* from developing to the adult stage, but not to the pupal stage. Thus, the cold treatment of citrus at ≤ 1.70 °C for 22 days may require additional consideration to be used as a standalone measure, unless agreed bilaterally or the use of lower temperatures in new confirmatory tests ensure complete larval mortality**.**

Keywords: *Bactrocera tau*, postharvest treatment, phytosanitation

Poster: Risk Assessment, Quarantine and Post-Harvest

Improved Quarantine System to Detect and Prevent the Introduction of Invasive Fruit Fly Species in Seychelles

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Background: Seychelles is highly dependent on food importation and hence increasingly exposed to high biosecurity risk for the introduction, establishment and spread of exotic fruit fly species. Offshore risk management and prevention of new fruit fly species introductions remain a major challenge faced by the country due to the increasing volume, complexity and speed of international trade, travel and tourism.

Until today, only two fruit fly species of economic importance are known to be present in Seychelles, the Mediterranean fruit fly (*Ceratitis capitata*), present since 1905, according the first record and the melon fly, *Zeugodacus curcubitae* (known previously as *Bactrocera cucurbitae*), introduced in 1999.

Methods: Given the importance of trade, travel and tourism of Seychelles economy, the government embarked on a strategic approach underpinning the main priority areas to boost up the quarantine system with strong combination of offshore risk analysis/assessment, certification scheme, inspection and verification, sampling, detection, identification, trapping network and emergency response plan.

These pathways provide the architecture for short to long term focus and consistency components with continuous effort to prevent the introduction, establishment and spread of exotic fruit fly species in Seychelles as the objective of the strategic intervention.

Results: Building the capacity of the targeted strategic areas has been improved with new equipment and materials, procedures and standards, technical know-how and training.

The interception of six different exotic fruit fly species from imported goods to Seychelles since 2000 are still reminders of the consequences that can significantly impair local production of fruits and vegetables: *Bactrocera zonata* in 2000, and *Ceratitis cosyra* and *Bactrocera dorsalis* in 2006 all in mango (*Mangifera indica*); *Dacus ciliatus*, twice in 2010 and 2013, respectively in zucchini (*Cucurbita pepo*) and musk melon (*Cucumis melo*); and in 2014 *Zeugodacus tau* (know previously as *Bactrocera tau*) in zucchini (*C. pepo*). In 2018, there was a consignment of pumpkin suspected to be infested with fruit larvae was intercepted. The consignment was destroyed and at the same time additional Tephri traps baited with either protein hydrolysate or terpenyl acetate were deployed around the airport area. Both fruit fly trapping and larval surveillance revealed the absence of any exotic fruit fly species.

Conclusions: As a result, no larval and adult fruit fly detection of invasive species has been identified in Seychelles despite the interception of some infested fruits by the quarantine services at the point of entry. This has contributed for maintain Seychelles as the only country in Sub-Saharan Africa (including Indian Ocean) free of *Bactrocera dorsalis.*

Keywords: Biosecurity, Fruit Fly Hosts, Detection, Inspection, Trade.

Poster: Risk Assessment, Quarantine and Post-Harvest

Plant essential oils as biopesticides for Caribbean fruit fly, *Anastrepha suspensa*

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Background: The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is a pest of over 100 fruit crops, including guava and citrus grown commercially in Florida, USA. Management of tephritid fruit flies often relies on bait sprays that incorporate synthetic insecticides. However, extensive use of these conventional insecticides has led to development of pesticide resistance in target species, contamination of the environment, and harmful effects on non-target organisms. Many plant extracted essential oils (EOs) have demonstrated toxic effects against insect pests, and therefore show promise as environmentally friendly alternatives to conventional insecticides. In this study, 18 EOs (representing six plant families: Apiaceae, Asteraceae, Cupressaceae, Lamiaceae, Myrtaceae, and Zygophyllaceae) were evaluated for toxicity against adult female *A. suspensa* to determine their potential as biopesticides.

Methods: GC-MS was used to analyse, isolate, and quantify the volatile chemical components collected from the plant EOs. Toxicity of each EO was determined using a topical bioassay. Aliquots of 1µg EO at different concentrations were applied to the dorsal thorax of adult females using a micro-applicator. Acetone was used as a control and all treated flies were kept overnight before scoring mortality results for data analysis.

Results: Of the18 EOs tested, 13 were effective toxicants against *A. suspensa* females, with varied median lethal dosage levels. In particular, the EOs from chamomile and juniper showed strong toxicities against *A. suspensa*. EO from Chinese chamomile showed stronger toxicity (LD_{50}: 7.58µg/fly) than EO from German chamomile (LD_{50}: 9.30µg/fly), and juniper EO extracted from fruit exhibited greater toxicity than EO extracted from leaves (10.45 and 22.07 µg/fly, respectively). Analysis of juniper oils indicated that α- and β-thujone were the most abundant components, and their toxicity in topical bioassays was similar to that observed with whole juniper oils.

Conclusion: EOs extracted from chamomile and juniper demonstrated the best potential as alternatives to conventional pesticides for control of *A. suspensa*. With juniper EOs, the α- and β-thujone constituents appeared to play a significant role in contributing to the toxicity of *A. suspensa*. Although more research is needed, this study provides useful information on the application of plant EOs for development of alternative management strategies for the Caribbean fruit fly.

Keywords: Tephritid fruit flies, essential oil, Caribbean fruit fly.

Poster: Risk Assessment, Quarantine and Post-Harvest

Post-Harvest non chemicals treatment Procedure for calibration of Vessels Approval

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Background: *In-transit cold treatment procedure for ships*: The use of sustained cold temperatures as a means of insect control has been used for many years. The vessels and/or containers must be capable of maintaining fruit pulp temperatures within the specific CT schedules.
Methods: *performance survey of vessels for approval*: Meeting with the ship's officer will provide better understanding of the blueprints of the ship and communication with the personal in the compartments. Operation check of temperature recording instrument: The instrument should be in operation for at least 30 minutes prior to calibration. Identification check of temperature sensors: Individual sensors must be checked to verify that they are properly labeled and correctly connected to the recorded temperature to determinate if the cables are correct as the position resistor plugs of specific sequential values (e.g., 29.5, 30.5, 30.0, 30.5, 31. F) are coupled to the cables.
Results: *calibration check of temperature sensors:* Compartment temperatures are to be lowered to near 0°C (32°F), for calibration test. *Ice/ water standards*. Mixture of fresh water is prepared in clean container. The ice must be crushed and completely fill the container. The ice water mixture must be carefully prepared and stirred to maintain a temperature of 32°F. The sensor must be submerged in the ice water mixture without touching the bottom of the container. testing each sensor In ice water continues until the reading is stabilized at the lowest temperature obtainable, two consecutives readings of the lowest temperature obtainable must be recorded on the temperature chart log sheet in order for calibration to be certified. Any sensor which reads more that plus or minus 0.3°C (0.5°F), from the standard 0°C (32°F) must either be corrected by calibration adjustment or replace, if none of this works, the officer should not certify the ship for doing in-transit cold treatments. report the complete details of the testing of the reading equipment and temperature sensors should be reported and fil all the technical information of the vessel

Conclusion: with this vessels calibration method, ships will be able to do a in-transit cold treatment without any delay, survey refrigeration, or sensors system failure that will impact in the effectivity of in transit cold treatment to control a quarantine pest.

Key Words: Methodology to do calibration in vessels, Record instrument, Temperature sensors, Ice water standards, testing the sensors.

Poster: Risk Assessment, Quarantine and Post-Harvest

QFly in a warming world: Biosecurity implications for Australia, New Zealand and globally

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Queensland Fruit Fly, *Bactrocera tryoni*, is Australia's most economically important horticultural pest. Recent climatic changes have allowed *B. tryoni* populations to overcome cold stress range limitations, expanding its range southward. This has undermined efforts to protect horticultural production in the Tristate area using the Fruit Fly Exclusion Zone (FFEZ), leading to its abandonment as a management tool. Recently *B. tryoni* was detected in Tasmania, and was the subject of an eradication campaign. Also, it has been detected many times in New Zealand and was the subject of successive eradication campaigns. However, it is unclear in all these cases whether in the absence of eradication efforts, the populations would have petered out naturally. Here we apply the CRU TS4 climate dataset to the CLIMEX model of *B. tryoni* to assess the geographical and phenological trends in climate suitability from 1970 – 2019. We found statistically significant trends in improving climate suitability at a range of sites in Australia and New Zealand. Additionally, we applied a business-as-usual future climate scenario to assess the sensitivity of the potential distribution in Australia, New Zealand and globally. We discuss the implications of the expanding potential distribution of *B. tryoni* for biosecurity and protection of market access in Australia and New Zealand and the global potential distribution.

Keywords: CLIMEX, *Bactrocera tryoni*, Qfly, niche modelling, pest risk analysis, distribution, climate change, seasonal phenology, invasion

Poster: Risk Assessment, Quarantine and Post-Harvest

TEPHRISURV: Development of a surveillance program for non-European fruit flies (Diptera: Tephritidae) in Belgium

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Background: The increased propagule pressure of non-European fruit flies into the European Union (EU) is considered a major risk to the European horticultural industry. Within the framework of the new EU Plant Health Law (Regulation EU 2016/2031) and the development of a list of priority pests (Delegated Regulation EU 2019/1702), EU members states are required to develop an action plan to avoid introduction of non-EU fruit flies. The Belgian FPS Public Health is funding a two years' project (TEPHRISURV) to the RMCA and ILVO to develop a fruit fly surveillance program specifically for the Belgian territory.

Methods: The TEPHRISURV project will tailor the list of 257 non-EU Tephritidae, compiled by the European Food Safety Authority (EFSA), to the Belgian situation. Based on an extensive literature study a shortlist of species that could potentially establish in Belgium will be compiled. Based on biological information of the selected species, combined with ecological modelling and information on the horticultural trade and activities in Belgium, possible sites of introduction and establishment will be determined. A surveillance programme will be developed specifically for Belgium.

Results: Two fruit fly categories can be distinguished: species able to become established in Belgium because of suitable conditions within the territory, and species for which the Belgian situation is not suitable but that can pose a risk to other EU countries through import and subsequent export. Based on the literature study and modelling data only one species of the four priority pests, listed by EU delegated regulation, might potentially establish in Belgium: *Rhagoletis pomonella*. In total 33 species are considered of medium/high risk to become established in Belgium, while an additional 20 species might be introduced into other EU countries via Belgium. A trapping and identification protocol is currently under development.

Conclusion: Fruit fly species that could potentially establish in Belgium can be largely subdivided into a group posing a risk to open field fruit production (mainly orchards of temperate fruits) and a group posing a risk to greenhouse production of solanaceous and cucurbitaceous crops mainly. The priority pest *R. pomonella* is posing a risk mainly for the first group. The other three priority pests (*Anastrepha ludens*, *Bactrocera dorsalis*, *B. zonata*) do not pose an immediate threat to the Belgian horticulture but could be accidently introduced through import and be further spread through subsequent export. Adjusted surveillance programmes need to be developed.

Keywords: quarantine species; monitoring; EU regulation, invasive species

Poster: Sterile Insect Technique

Assessment of isotopic enrichment method for persistent marking of Queensland fruit fly for use in sterile insect technique

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Background: Discrimination between wild and sterile flies is pivotal to success of sterile insect technique. Stable carbon isotope ratios are proposed as a precise tool to discriminate between wild and sterile adults of Queensland fruit fly (Q-fly), *Bactrocera tryoni* (Froggatt), the most devastating pest of horticulture crops in Australia. A previous study demonstrated successful use of natural $\delta^{13}C$ value to discriminate wild and sterile Q-fly but with limited knowledge about persistence of the $\delta^{13}C$ value once the released sterile adults start foraging on C3 plants in the field.

Methods: We conducted an enrichment study by incorporating commercially available ^{13}C glucose in gel larval diet to understand changes in isotopic incorporation into body tissues following metamorphosis. We seeded Q-fly eggs onto each ^{13}C D-Glucose enriched and control diet and compared the changes in isotopic values at different pre-imaginal stages. Pupae from each enriched and control diet were allowed to emerge as adults and the emerged adults were provided a standard 3:1 diet of sugar and yeast hydrolysate. The adult flies were collected at different ages (0, 5, 10, 30 and 60 d) and change in their isotopic values were compared.

Results: Our results suggest that the ^{13}C isotope enrichment through larval diet is incorporated and fixed into body tissues and retention of the isotopes is at a level significantly higher than the control at all the pre-imaginal and adult stages allowing precise discrimination of the enriched flies from the control. The persistence of carbon stable isotope ratios from the enriched larval diet spiked with a low concentration of ^{13}C glucose retained through Q-fly adult life. There was a significant depletion of $\delta^{13}C$ value as the flies aged, however, even when the flies reached 60 d old the $\delta^{13}C$ value of the flies from enriched larval diet were significantly higher than the control files.

Conclusion: Stable isotope enrichment can be used to complement stable isotope marking based on natural abundance. Laboratory studies of the present study need to be extended to assess the persistence of ^{13}C isotopic values of the flies when they are released to the field.

Keywords: Tephritidae; *Bactrocera tryoni*; Body tissue; Carbon; Pre-imaginal stages

Poster: Sterile Insect Technique

Bacterial diversity associated with Anastrepha obliqua males change under mass-rearing conditions and with irradiation

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Background: The intestinal microbiota in tephritid fruit flies plays an important role in the development, physiology, homeostasis and performance of their hosts. The use of symbiotic bacteria in fruit fly programs employing the sterile insect technique (SIT) has the potential to increase the sexual competitiveness of insects sterilized by irradiation. Our objective was to compare the bacterial communities in the midgut of wild and mass-reared *Anastrepha obliqua* (Macquart) males irradiated with different doses.

Methods: Pupae of wild and mass-reared *A. obliqua* were irradiated with 0, 60, and 80 Gy. After adult emergence, their midguts were dissected, DNA was extracted, and high throughput sequencing of the V4 region of the 16S rDNA gene was performed.

Results: A total of 11 phyla, 17 classes, 47 families, and 52 genera of bacteria were identified. The most representative phylum was Proteobacteria and the predominant family was Enterobacteriaceae. We found that wild males had a different intestinal bacterial community than mass reared males. In addition, irradiation at 60 and 80 Gy caused changes in the diversity and structure of the midgut microbiota of these sterile males, suggesting that mass rearing and irradiation cause artificial selection of the bacterial communities in the gut of *A. obliqua* males.

Conclusion: We found marked differences between the midgut microbiota associated with wild males and mass reared males irradiated at different doses. These differences suggest that mass rearing processes have a strong effect on the diversity and abundance of gut bacterial communities. Irradiation also produces changes in the diversity and structure of the bacterial communities in the midgut of *A. obliqua* males, but of lesser magnitude.

Keywords: Symbionts[1], West Indian fruit fly[2], Sterile insect technique[3], Irradiation[4], Gut microbiota comparison[5], Bacterial communities[6]

Poster: Sterile Insect Technique

Control of *Bactrocera dorsalis* (Diptera:Tephritidae) using Sterile Insect Technique in Phetchaburi Province

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Background: The Department of Agricultural Extension (DOAE) supported Sterile Insect Technique (SIT) integrated with other environmental friendly methods to control fruit flies in Trok Nong sub - district, Khlung district, Chanthaburi province with suppression strategies since 2013, and in collaboration with TINT since 2018 to present. The wild fruit flies population in the treated area have been decreased more than 90% compared to untreated area. Chemicals application cost for fruit flies control has been reduced, damage fruits from fruit flies infestation have been reduced and SIT has been accepted. The positive results induced DOAE decided to expand SIT to support the area of GI (Geographical Indication) product plantation, Rose apple (*Eugenia javanica* Lam.) the main economic crop of Phetchaburi province which cover some part of Nong Sano sub - district, Ban Kum sub - district, Mueang Phetchaburi district, and Tha Raeng sub - district, Ban Laem district Phetchaburi province.

Methods: Since August 2019, the target area was mapped as 2,747 Rai (439.52 ha) core area, and 6,771 Rai (1,083.36 ha) buffer zone using GPS and GIS under area-wide concept. Fixed points of surveillance trapping system using steiner trap were marked 10 points in core area, and 7 points in buffer zone followed the international standard for wild population monitoring. Traps were serviced weekly continuously and fly per trap per day (FTD) were calculated. Integrated pest management such as male annihilation technique (MAT), alternative host removal, bagging etc. were applied including orchards sanitation. In 2020, mass traps making technique was transferred and equipment were supported for project participatory growers. IPM were applied continuously, especially infested fruits were collected and transformed as biofertilizer. Traps service was carried out weekly while fruits sampling was conducted twice a month. During March - August 2021, sterile flies of *Bactrocera dorsalis* GSS white - thoraxed which was developed under collaboration of DOAE and TINT between 2016 - 2019 and former supported in Chanthaburi province were continuously released every two weeks. In 2022, for more efficiency of SIT - AWIPM, sterile flies were weekly released during April - June.

Results and conclusions: Sterile Insect Technique is the contraceptive method to control fruit fly, proactive biotechnology, environmental friendly and sustainable. Released of *Bactrocera dorsalis* GSS white - thoraxed has been increased the efficiency of Sterile Insect Technique in integration with other methods. Wild fruit fly population has been decreased continuously since 2019, 2020, 2021 and 2022. Applications of chemicals for fruit fly control have been reduced and SIT has been accepted.

Key words: Geographical Indication, *Bactrocera dorsalis* GSS white-thoraxed, sustainable, SIT-AWIPM

Poster: Sterile Insect Technique

Do pre-release caffeine supplements promote sexual performance in *Bactrocera dorsalis*?

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Background: The sterile insect technique is a powerful method of controlling populations of various insect pests including *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). On the other hand, male annihilation using methyl eugenol and malathion, or similar synthetic insecticides is a common practice which is very popular among crop protectionists because of its success rate. There is growing interest to deploy these two techniques simultaneously, but the concern is on irradiated flies being attracted to the methyl eugenol traps. Males of *Bactrocera dorsalis* consume methyl eugenol in nature from plants and synthesise the compound into a pheromone which enhances mating success with females. Experiments elsewhere have shown that feeding caffeine to the Queensland fruit fly (*Bactrocera tryoni* Froggatt) promoted sexual maturation and they postulated that similar benefits could be possible in other fruit flies.

Methods: *Bactrocera dorsalis* pupae were placed in the Gamma radiation source (Cobalt-60) chamber and irradiated with 70Gy radiation dose, 2 days before eclosion. Assuming a pre-holding period of 3 days, flies were fed different types of caffeine sources mixed with sugar and yeast enzymatic hydrolysate in a ratio of 3:1. The caffeine sources included analytical caffeine and two other Kenyan ground coffees. These were analysed for caffeine content, metabolites, and macromolecules before use. In all cases, the concentration of caffeine was 1, 3, and 5%. After the 3-day feeding period, flies were transferred to sugar only diet and thereafter assayed as required. We tested the survival of the flies post-feeding, time is taken to mate, duration of mating, and the size of the ejaculatory apodeme, testis, and accessory glands. Their response to methyl eugenol post-feeding was also evaluated.

Results: Analytical caffeine had the highest caffeine content and quantities of metabolites and macronutrients varied significantly among the tested sources. Experiments are still ongoing, but it is evident that caffeine enhances sexual performance, and may be used used as a pre-release supplement for *B. dorsalis*. We discuss our findings in the context of simultaneous application of SIT and male annihilation.

Conclusion: Pre-release supplements which confer enhanced competitiveness of flies are ideal in the simultaneous application of SIT and male annihilation. This will certainly reduce cost and result in a greater effect of the two techniques when combined than when implemented separately. Caffeine is readily available especially in Africa from the production of coffee and cocoa. The mode of administering through mixing with sugar and yeast hydrolysate is feasible and may be improved if need be.

Keywords: *Bactrocera dorsalis*, Caffeine, SIT, Male annihilation, Sexual performance, Irradiation

Poster: Sterile Insect Technique

Effect of humidity on the survival of sterile *Bactrocera correcta* (Diptera: Tephritidae) males in the field

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Background: Survival of sterile fly in the field after being released is very relevant and contributes to the success of Sterile Insect Technique (SIT) method for management. However, similar to other insects, sterile fly's lifespan depends not only on their quality but also upon factors from nature including temperature, humidity, food, natural enemies,…etc. An evaluation on rearing sterile *Bactrocera correcta* in diffirent humidity regimes is implemented to study an effectively sustainable control in SIT for this species.

Methods: The *Bactrocera correcta* flies used in this study was recovered from the same size pupae, 12-12.3mg and radiated at 80Gy using a Co60 grammacell 2 days before emergence (age of pupae was determined by the change in eyes' color). Five different humidity regimes were 55%, 60%, 75%, 80% and 85% RH. Each treatment was repeated three times while there will be one cage with one hundred 1-day-old sterile males. Cages were kept in incubator at a temperature of 28°C and photoperiod 10: 14 hours (light: dark). We checked and recorded on a daily basis for lifespan of sterile male.

Results: There was a difference in the lifespan of sterile *B. correcta* males when being cultured in different humidity regimes.: Under rearing conditions of 28°C temperature and 55% RH, the average life expectancy of male is 30.0 days. Whereas under the same conditions of temperature with varied level of humidity of 60%, 70%, 80%, 85% RH, it was recorded as 31.8; 31.7; 24.9; and 21.0 days respectively. The high humidity of 80 and 85% RH had a significant negative impact on the lifespan of the adult sterile males.

Conclusions: Lifespan of sterile *B.correcta* male is strongly affected by humidity. This should be noticed when handling and releasing sterile insects for SIT in the field.

Key Words: Bactrocera correcta, release, survival.

Poster: Sterile Insect Technique

Effect of limited protein consumption during the larval and adult stage on life-history traits of the Mexican fruit fly

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Background: *Anastrepha ludens* Loew (Diptera: Tephritidae) is an important mango and citrus pest. The sterile insect technique (SIT) is currently used to control this fly. The SIT involves the release of sterile insects to reduce wild populations through the induction of sterility. Prior to release, insects are mass-reared on artificial diets. The rearing of *A. ludens* involves the provision of protein both in the larval and adult stage, thus we studied trade-offs between larval and adult diets.

Methods: We compared various yeast concentrations in the larval diet and two formulations in the adult diet, both at small and semi-mass scale. We determined the quality of the insects at all developmental stages.

Results: Low concentrations of torula yeast (TY) (2.9 and 3.6%) produced larvae and pupae of low weight, fewer larvae and pupae, and more males than higher TY concentrations (6.1%), but this was only discernible at a small scale. Adult survival without water and food was significantly lower for non-irradiated males and females, and irradiated males when reared on 6.1% TY. For mating success, we found that non-irradiated males reared on 4.8% TY but fed only sugar (S) as adults had lower probability of mating than males fed yeast hydrolysate (YH). For irradiated males, larval diet had no effect on mating competitiveness, only males fed with S+YH in the adult diet had higher mating success than those fed with S only.

Conclusion: We recommend reducing TY in the larval diet to 5.1%; however, for release colonies S+YH will need to be provided in the adult diet. Knowledge on larval and adult nutritional requirements will aid in our understanding of insect nutritional ecology and will also improve mass-rearing diets.

Key Words: *Anastrepha ludens*, artificial diets, Diptera, mango and citrus pest, mass rearing, SIT, sterile insect technique, Tephritidae, trade-off, yeast hydrolysate.

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Females of the melon fly, *Zeugodacus cucurbitae*, mate indiscriminately with males from a laboratory and wild strain (Diptera: Tephritidae)

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Background: The melon fly, *Zeugodacus cucurbitae*, is a global pest of many commercially important fruits and vegetables. The Sterile Insect Technique (SIT) has been used to control the species, most notably in the Ryukyu Islands of Japan. The SIT involves the release of mass-reared, sterile males to obtain matings with wild females, resulting in the production of inviable eggs and eventual population reduction. The ability of released males to compete successfully against wild males determines the success of the SIT. However, artificial selection acting under mass-rearing conditions may alter male behavior in ways that decrease acceptance by wild females. A pupal-color sexing strain of melon fly (termed T1) was developed in Hawaii, and evidence suggests that, even after 20 years of laboratory rearing, males of this strain are equal to wild (W) males in mating competition. The present study describes two experiments that confirm this prior result.

Methods*:* In the first experiment, trials conducted in field cages assessed the impact of two elements - overflooding ratio (i.e., T1:W males) and use of male-only vs. bisexual releases of T1 flies – on the mating success of T1 males. Four overflooding ratios - 1:2, 1:1, 2:1, 10:1 – were established, each of which was evaluated with and without the release of T1 females. Eight trials were conducted for each of the 8 treatments (four overflooding ratios each with T1 females present or absent). In the second experiment, male-female pairs (T1♂ x T1♀, T1♂ x W♀, W♂ x W♀, and W♂ x T1♀) were observed in the laboratory, and the incidence and duration of male wing-fanning as well as the incidence of mounting and mating were recorded.

Results: At all overflooding ratios and independent of the presence of T1 females, observed matings between T1 and W flies did not differ from those expected by chance. Male-female observations revealed that T1 and W males displayed similar levels of wing-fanning, and both the incidence and success of mountings were similar across the different pairs.

Conclusions: The present study indicates that *Z. cucurbitae* females from a genetic sexing strain or a wild strain mate randomly with males from these same two strains. The high mating ability of T1 males suggests that this strain could be used effectively in SIT programs. Nonetheless, other parameters, such as survival and dispersal, require further evaluation.

Keywords *Zeugodacus cucurbitae*, Sterile Insect Technique, Random Mating

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Implementation of the Chilled Adult Release System for releasing of sterile insects on the Fruit Fly Control and Eradication Program - Argentine

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Background: the Mediterranean fruit fly (Ceratitis capitata Wied.) is a key pest for the development of the main fruit-growing areas of Argentine Republic. Mendoza Province and Patagonian Region have the largest fruit area in Argentina of stone and pommes fruits, in a highly favorable agro-ecosystem for the production of high-quality fruits with excellent sanitation. The presence of Mediterranean fruit fly restricted the markets, due to the limitation of exportation to countries with quarantine restrictions. On 1994, Argentina implemented the National Fruit Fly Control and Eradication Program (PROCEM) with the proposal to use "IPM", with emphasis on the use of the Sterile Insect Technique. The development of the SIT has its center for mass rearing operations at Mendoza Province. Since the beginning of the program (1992), packaging system with paper bags and dry food was implemented. Based on the availability of new technology "Chilled Adult Release System", developments, adjustments and field validations was implemented since 2017.
Methods: the methodology focused developing all aspects related to this technology to cover a release module of 100 million sterile ♂/week (TSL - VIENA 8$^{(-INVD53)}$ *GSS*),

- Strategic analysis of the best location
- Adequate building infrastructure
- Acquisition of specific equipment/air conditioning
- Analysis, development and validation of the logistic for flights.
- Development and implementation of weather prediction models
- Development of release aircraft for the region
- Training of human and professional resources.

Insect quality measurements were made post irradiation, post transportation, post cooling, post release, flight times, ferry times and the main field parameters related to mating behavior, competitiveness, survival, dispersal, recapture, among others.
Results: As summary of results, the chilled adult packing center is located on Gral. Alvear (Mendoza). CESSNA 180/TECNAM 2006 were aircraft used, with dynamic boxes of 10 million each. The average quality parameters achieved, were: pupal weight 8.19 mg; % Survival under stress (48h) 90.99 %, Emergency 91.91%, Flying 84.97%. The area under release 14,300 has for Mendoza and 13,160 has for Patagonian Region. The release times ranged in each zone between 90 min and 120 min, with dynamic blocks according to the recapture observed, and ranging between 1000-3500 ♂ Vol/ha. Recapture levels showed a substantial increase in sterile *FTD* and distribution improved.
Conclusions: the use of chilled adults system has been adapted and verified in the field, achieving better sterile male's quality, hydration and feeding prior to release. Good acceptance of the technology by growers, substantial improvement in the quality of the insect, competitiveness increased, increasing of recapture rates and better distribution, a positive cost-benefit ratio compared to the traditional technique of paper bags.

Keywords: Mediterranean fruit fly, chilled adults release system, Sterile Insect Technique

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Mass-rearing characterization of the *Ceratitis capitata* (Diptera: Tephritidae) faster development female temperature sensitive lethal genetic sexing strain

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Background: The white pupae (*wp*) and temperature sensitivity lethal (*tsl*) genes are two selected markers used for the construction of the genetic sexing strain (GSS) of the Mediterranean fruit fly (*Ceratitis capitate*) (Wiedemann) (Medfly) Vienna 8^{D53-}, which allows the production of male-only pupae. In this strain, females emerge from *wp* pupae and are temperature-sensitive, while males emerge from wild type pupae. When eggs are exposed to a high temperature (34°C), the female embryos die, allowing only males to be produced. The development of the females from the original Vienna 8^{D53-} *tsl* is slower during the larval stage. Male larvae pupate two to three days earlier than female larvae. Recent research has shown that different recessive genes regulate the slow development of larvae. Fact which allows the creation of a novel strain with faster development female (FDF). We report the results of experiment in which the mass rearing a quality control characteristic of the new Vienna 8^{D53-}FDF strain was compared with the conventional Vienna 8 $^{D53-}$.

Methods: The comparison of the two strains was done using rearing and quality control protocols for the mass-production of Medfly GSS during nine consecutive generations. In addition, mating compatibility and mating competitiveness between Vienna 8^{D53-} FDF and the Türkiye wild strain were determined in a greenhouse field cage.

Results: Vienna 8^{D53-} and Vienna 8^{D53-} FDF produced similar numbers of eggs per female per day for the entire experimental period. However, the fertility of the Vienna 8^{D53-} FDF was significantly higher than the egg hatch of the Vienna 8^{D53-}. The emerge of a male in the Vienna 8^{D53-} FDF strain was significantly higher than the emerge of male in the Vienna 8 $^{D53-}$strain. On the other hand, there was no noticeable difference between Vienna 8^{D53-} FDF and Vienna 8^{D53-} strains for the percentage of female emergence. The *wp* was obtained at the second and third pupal collections in the Vienna 8^{D53-} FDF, at the fourth and fifth pupal collections in the Vienna 8^{D53-} strain. In the field cage, The competitiveness of the Vienna 8^{D53-} FDF males, irradiated with 120 Gy were slightly lower than the wild males from Türkiye in mating with Türkiye females.

Conclusion: In the current study, the Vienna 8^{D53-} FDF showed equal or better parameters utilized the measure the production and quality profile of both strains. However, the collection of *wp* in early pupal collection days provided an advantage in terms of space, energy savings and overall production cost. Therefore, making more efficient SIT application against Medfly.

Keywords: Medfly; mass rearing; mating competitiveness; wild strain

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Natural source of Methyl eugenol and sexual performance of *Bactrocera correcta*

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Background: Bactrocera correcta colony has been established in the Plant Protection Research Institute (Viet Nam) since 2008. However, living in the artificial habitat for a long time can result in being less competitive at mating. Therefore, there is a need for improving the quality of *B. correcta* by supplying a suitable adult diet, which will allow us to develop a new rearing procedure of *B. correcta* for management.
Methods: Leaves of *Ocimum tenuiflorum* are dried by the scattered light for seven days, and then blending into powder; finally, it is mixed with the adult diet at 10% (ME diet). Male of *B.correcta* are fed in six following treatments: Sugar (T1); Protein (T2); Protein plus Sugar (T3); Sugar plus ME (T4); Protein plus ME (T5); Protein plus sugar plus ME (T6). A newly emerged male was released in cylindrical cage (8cm diameter x 30cm high) at 16:30, supplied with water through a wet sponge, and fed by each of the six different treatments of adult diet. Thirty males were tested per replicate with 3 replicates for each treatment. One 20-to-30-day-old virgin female was added after thirty minutes and observed. Females used in the experiments were sexed on the first day of adult life and reared in screen cages (30cm x30cm x30cm) without exposed to males. They were provided with a sugar plus hydrolyzed yeast (3:1) and water. The data on mating were recorded. The same procedures were repeated daily until males were 40 days-old.
Results: The earliest mating occurred on day 5 for males in T3 and T6, on day 7 for males in T1 and T4. Males in T2 and T5 died completely at 4 days-old and no mating activity was observed. The rate of mating pairs in T3 and T4 was approximately 57.8% and 76.7%, respectively and it obtained more significantly than males of T1 at statistical significance $P \leq 0.05$ (47.8%). The males from T6 was the highest percentage of mating success (78.9%).
Conclusions: This study asserts the role of Methyl eugenol in repeated mating behavior of *B.correcta* and suggests using powder of *Ocimum tenuiflorum* leaves as a solution to replace Methyl eugenol chemical to bring more benefit in mass-rearing.
Keywords: Bactrocera correcta, mating behavior, *Ocimum tenuiflorum*.

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Overwintering of Mediterranean Fruit ly Adults in Dalmatia and Implications to Current Strategy of SIT Suppression Program in Neretva Valley

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Background: Suppression of Mediterranean fruit fly or medfly, *Ceratitis capitata* Wied. (Diptera, Tephritidae) in the Neretva valley integrate Sterile Insect Technique (SIT) with other control methods. The release of sterile medfly males begins in the late April and continues through November. This strategy was developed 15 years ago when an area-wide control program supported by FAO/IAEA was initiated. The release strategy was based on available data that the pest was overwintering only as larvae in the fruit of host plants at that time.
Methods: In order to optimise the release strategy, we analysed available data from annual reports for 2014-2017 on medfly overwintering in the Neretva valley: i) emergence of overwintering larvae from infested mandarin fruits during winter, ii) detection of adults at hotspots from April to June and iii) available and suitable host plants fruits in late winter. In addition, we analysed the above data and complement it with results of experiments on overwintering of adults conducted as a part of the EU-funded project FF-IPM ("*in-silico* boosted, pest prevention and *OFF-season* focused IPM against new and emerging fruit flies" http://fruitflies-ipm.eu) during winter and spring 2019-2021.
Results: Multi-year data from the Neretva Valley confirm that the medfly overwinters mainly as a larva in fruits, followed by pupation in the soil and emergence of adults during April and May. Several captures of adult females in 3C lure traps were recorded during early April at hotspot locations in open fields, as well as findings of fully developed larvae in kumquat, *Fortunella japonica* in early April. Experiments on the *OFF -season* survival potential of adults fill in the gaps in our knowledge of medfly overwintering capacity, showing that a small percentage of adults can survive through April in open fields, while adults in basements in urban areas remained alive through May. In most experiments, females outlive males.
Conclusion: Most of the medfly population overwinters as larvae in fruit, and adult emergence least during April and May. Recent experiments show that a small but very important percentage of adults are able to overwinter in both open field conditions as well in protected spaces such as basements in urban areas. Mandarins and fresh fruits of kumquat represent quality hosts in which the pest will reproduce and establish a population in the early spring. Basements in urban areas are additional hotspots for medfly overwintering. Therefore, the release strategy should be optimized by earlier release of sterile males concentrating on hotspots in open fields and urban areas.
Keywords: Medfly, Sterile Insect Technique, overwintering,

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Quality control traceability during the packing and release process of sterile Ceratitis capitata for SIT purposes

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Background: In programs applying the Sterile Insect Technique (SIT), the quality of the insects deployed in the field determines the success in suppressing, containing, or eradicating the pest population. In the emergence and release facility (ERF) of the Moscamed Program in Mexico, irradiated pupae of Ceratitis capitata are packed in Mexico-type towers and key quality parameters, such as adult emergence, adult fliers, and adult survival, are determined throughout the packing and release process. However, different methodologies are used to estimate the percentage of adult fliers in the different stages of the process; raising reasonable doubts of whether observed differences are due to the effect of each stage or to the used methodology.

Methods: We developed an alternative called “Adult Flier device” (= AF-device) to evaluate the adult flier parameter following a critical evaluation path of five steps: 1) upon arrival, 2) post-packing, 3) post-holding, 4) post-chilling, and 5) post-release, where adult fliers and survival under stress were evaluated. We also compared the current methodologies for the estimation of "absolute fliers" available in different operating manuals.

Results: Our results suggest that the AF-device allows reliable traceability of sterile insect quality parameters throughout the packing and release process, since no significant differences were observed with the control treatments. In the chilling stage, the five methodologies tested were equivalent, but the AF-device resulted less time-consuming and required less work force and biological material than the other methodological options.

Conclusions: Our results demonstrate that the use of the AF-device can be a feasible, versatile, innovative, and efficient alternative to evaluate quality control parameters throughout the process of packing and releasing sterile insects, providing reliable results in a timely manner with less work and using minimal biological material.

Key words: Fruit flies**,** emergence and release facilities, adult fliers, AF-device.

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Recent contribution of the Insect Pest Control Laboratory to the development of the Sterile Insect Technique for *Drosophila suzukii*

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The Insect Pest Control Laboratory (Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture) (IPCL) carries out Research and Development activities that aims at improving the use of the Sterile Insect Technique (SIT) for major insect pests of agriculture, veterinary and human importance. Over the recent years, the IPCL has made significant progress in the development of SIT methods adapted to the Spotted Wing Drosophila (SWD), *Drosophila suzukii*, an invasive fly pest that attacks soft fruits and causes severe economic losses worldwide. We propose here a concrete overview of the different tasks achieved or under investigation at the IPCL in terms of mass-rearing, irradiation, and quality control procedures, in the light of other advances accomplished by Member States.

Key Words: Spotted Wing Drosophila, Sterile Insect Technique, International Atomic Energy Agency

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Reduced activity levels and increased sleep fragmentation in laboratory vs wild populations of *Bactrocera oleae* (Rossi) adults

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Background: Olive fruit flies, *Bactrocera oleae* (Diptera: Tephritidae) that are laboratory reared on artificial diets are essential for the genetic control techniques for this pest. However, the colony's laboratory adaptation can affect their quality. The aim of this study was to compare the activity and sleep patterns of a wild and a lab-adapted population of olive fruit flies through the detailed tracking of their locomotor activity with the Locomotor Activity Monitor.

Methods: Individual flies were housed inside each of 32 tubes (25 mm diameter, 125 mm length) of the Locomotor Activity Monitor (LAM25, Trikinetics, MA, USA) under constant laboratory conditions (24±1.5 ºC, RH 30±5%, Light:Dark 14:10h). Organdie cloth covered one end of the tube for ventilation, while the opposite end was closed with a rubber cap containing agar gel providing food and water. Tubes are vertically crossed by infrared beams. Counts of beam breaks caused by the fly activity are used as an estimation of its locomotor activity levels during the 14h of photophase (day activity) and during the 10 h of scotophase (night activity). More than 5 minutes of inactivity (zero beam counts) were considered as one sleep episode. We used olive fruit flies of a wild population reared on olives for no more than 2-3 generations and of laboratory adapted flies reared in artificial for more than 300 generations. Both sexes were monitored at a young age (2-3 days old) and at sexually mature age (10-12 days old).

Results*:* Day activity levels were higher for young wild females compared to young wild males, but sexually mature wild males were had higher day activity levels than the wild females of the same age. Wild flies of both sexes had minimal night activity at the tested ages. Lab-adapted flies had higher night activity than the wild ones, both at young and mature ages. During the scotophase, lab-adapted flies had more sleep episodes compared to the wild flies and of lesser mean duration, i.e a more fragmented night sleep pattern. The day activity levels of lab-adapted males were lower compared to the wild males. The latter also had more sleep episodes during the photophase than the wild ones.

Conclusions: Laboratory adapted colonies are expected to produce more sedentary flies, due to the rearing conditions. Indeed, we showed that lab-adapted olive fruit flies had lower day activity levels than the wild. Additionally, we found that lab-adapted flies had fragmented night sleep compared to the wild ones. Poor sleep quality may affect the ability to survive, courtship wild females and compete with wild males thus being a disadvantage for lab males.

Key Words: olive fruit fly, locomotor activity, laboratory-reared, Sterile Insect Technique

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Remating in *Bactrocera dorsalis* males and females: implications in sterile insect technique programmes

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Background*:* Sterile insect technique (SIT) is a sustainable biological tool for managing fruit fly species (Tephritidae). This technique is considered against *Bactrocera dorsalis* in the Reunion Island, where this pest triggers severe economic impacts, especially on mango production. However, detailed knowledge of bioecology is necessary to consider this technique, model it and determine the release parameters (frequency, quantity...). In this study, we focus on the mating behaviour of *B. dorsalis.* In Tephritidae, mating behaviour is complex because males are aggregated in lek to attract females, and females actively choose a mate from these regrouped suitors. Laboratory mass-rearing and sterilisation can result in changes in the behaviour of males and their mating capacities.

Methods: We investigated the remating behaviour of males and females under laboratory conditions. We used three types of males: i) fertile wild males (larvae collected in field fruit), ii) fertile laboratory males, or iii) sterile laboratory males, that were sterilised by the IAEA, Vienne from a lab strain of *B. dorsalis* from la Réunion. We compared the proportion of females that remated and the duration of the refractory period (from 1 to 28 days) according to the type of male proposed to the female for the first mating. In addition, we investigated differences in frequencies of male remating during one week according to the type of males. Then, we determined whether sperm transfer was efficient by dissection of mated female spermathecae.

Results: Males remated many times consecutively without a refractory period, and sterile and laboratory males remated significantly more than wild males. Under our laboratory condition, only 11 /70 females remated from 1 to 7 days after the first mating. This proportion was not influenced by the origin of the male (sterile, lab or wild) during the first mating.

Conclusion: In conclusion, our results may have important practical implications for area-wide management strategies for *B. dorsalis*. Our estimated remating parameter could be integrated into the model to determine the number and frequency of sterile male releases.

Keywords: sterilized males, mating, sperm transfer

Poster: Sterile Insect Technique

Republic of turkey ministry of agriculture and forestry
Title: studies on biotechnical methods against mediterranean fruit fly and transition to sterile insect release studies in türkiye
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One of the exotic fruit flies which is Mediterranean fruit fly (Medfly) [*Ceratitis capitata* Wied. (Diptera: Tephritidae)] is one of the significant pests of fruits cultivation areas in Türkiye being polyphagous and quarantine organism. Its tolerance is considered to be zero due to the possibility of transmission to exporter countries by means of contaminated fruits. Implementation of an effective control method against the Mediterranean fruit fly is one of the priority issues because of increasing trend in citrus export from Türkiye.

In Türkiye, the first studies of SIT started in the 1960’s. Later, between the years 1973 and 1982, project studies have been held with the support of International Atom Energy Agency. Steril medfly released on semi-isolated 20 decar citrus fields in Cesme District of Izmir Province in 1980 and 1981. In the field controls made in 1982, the studies were detected as 80% successful and the project was finished (Zumreoglu et al,1982).

Uses of biotechnical control is increasing in generally bait spray is the most common control measure against Medfly in Türkiye. Steril Insect Release Technique (SIT) that provides better suppression of the pest in area-wide control programs than chemical control becomes even more important.

It is planned to put SIT into practice in area wide Medfly suppression programs enhancing its efficiency in citrus growing areas in Menderes of Türkiye, between 2023-2025 years. In case of being successful, large-scale control programs will be implemented to suppress the pest in wider areas including different hosts plants in Türkiye..

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Status of the tephritid fruit flies production worldwide for the sterile insect technique application

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Background: This review presents the data related to the facilities worldwide producing sterile fruit flies of economic importance for the sterile insect technique (SIT) application. The data includes production capacity, types of irradiator and sources, and the sterilization doses used.

Methods: This data was mainly compiled from two databases that are kept up to date (1) DIR-SIT (the World-Wide Directory of SIT Facilities), and (2) IDIDAS (the International Database on Insect Disinfestation and Sterilization).

Results: Presently, ten different tephritid species are under production for SIT operational programmes. Facilities producing sterile tephritid fruit flies are leading in terms of the number of sites, with 23 facilities around the world, and the production volume with over 3 billion sterile fruit flies per week. For most tephritid fruit fly species, doses between 80 and 140 Gy are sufficient for producing good quality sterile flies.

Conclusion: The status of the worldwide production and sterilization of tephritid insects provides a global perspective and highlights the importance of the SIT area-wide application for insect pest control.

Keywords: Tephritidae, fruit flies, sterile insect technique, irradiation, mass-rearing facilities

Poster: Sterile Insect Technique

Trade-off between duration of pupal hypoxia for irradiation and transportation and quality control parameters of sterile Queensland fruit fly *Bactrocera tryoni* (Diptera: Tephritidae)

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Background: The sterile insect technique (SIT) is used in area-wide integrated management of Queensland fruit fly (Q-fly) *Bactrocera tryoni* (Froggatt), as well as to eradicate outbreaks in fruit-fly-free regions. Before exposure to ionising radiation to induce sterility, Q-fly pupae are usually sealed in plastic bags to create hypoxic conditions that have a crucial role in minimizing irradiation damage. Post-irradiation, the sterile insects are transported to distant locations still under hypoxia which may diminish fly quality. To date, studies investigating the effect of hypoxia on fly quality have focused on emergence rates and have indicated modest reductions. A different view emerges in the present study that includes a complete analysis of fly quality.
Methods: We investigated the effects of 0, 1, 2, 4, 6 and 8 days of storage of irradiated pupae under hypoxia at 18°C or 25°C. In addition to assessing standard quality control parameters, a locomotor activity monitor device was used to assess supplementary parameters including pre-emergence period and activity under nutritional stress.
Results: Adult emergence was not affected up to 2 days of hypoxia and pupae stored at 18°C had a higher emergence rate than those stored at 25°C. Flight ability remained at acceptable levels when pupae were stored for up to 2 days at either temperature but was substantially reduced when pupae were stored for 4 days or longer.
Conclusions: Q-fly pupae can safely be exposed to hypoxia for up to two days. Longer periods of hypoxia should be avoided, but if required should be at 18°C rather than 25°C. Procedures that enable longer time frames for irradiation and transportation of pupae to rear-out facilities while maintaining fly quality would be of significant operational value in Q-fly SIT programs.
Key words: Hypoxia, Emergence, Flight ability, Locomotor activity monitor, Sterile insect technique

Poster: Natural Enemies and Biological Control

Differential immune response in new and old fruit fly-parasitoid association: Implication for their management

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Background: *Bactrocera dorsalis* (Hendel) and *Ceratitis cosyra* (Walker), are major fruit-infesting tephritids across sub-Saharan Africa. Biological control of fruit fly pests using parasitoids has been widely used but with varying levels of success. Most studies investigating host-parasitoid interaction have focused on the functional and evolutionary aspects leaving a knowledge gap about the physiological mechanisms underpinning the efficacy of parasitoids as biocontrol agents. This study sought to establish the nature and magnitude of the cellular immune responses of *C. cosyra* and *B. dorsalis* when exposed to the larval parasitoids, *Diachasmimorpha longicaudata* (Ashmaed) and *Psyttalia cosyrae* (Wilkinson) (both Hymenoptera: Braconidae).
Methods: Two groups of 100 second-larval-instar of C *cosyra* and *B. dorsalis* were placed in oviposition-units containing carrot-based-diet. One group of each host larvae was separately exposed to *D. longicaudata* or *P. cosyrae* held in separate cages. At 24 and 36-h post-exposure, larvae were dissected under a stereo microscope and occurrence of encapsulation/melanisation of the parasitoid eggs was recorded. Furthermore, the hemolymph of the parasitised and non-parasitised host larvae was extracted and used for immunological analyses; total and differential hemocyte counts, viability, and spreading of hemocytes were recorded. In another set of experiment the parasitised larvae were maintained until host and/or parasitoid eclosion and percent parasitism of each host-parasitoid combination was computed.
Results: Non-parasitized *B. dorsalis* had up two-fold of total hemocyte counts (THC) as that of *C. cosyra*. This trend was also observed post-exposure to parasitoids. *Bactrocera dorsalis* had a higher proportion of viable hemocytes with a higher spreading ability. In terms of differential haemocyte counts (DHC), plasmatocytes were the most abundant in both host species pre and post-parasitisation by either species, even though exposure to parasitoids reduced plasmatocyte counts. However, parasitisation increased granulocyte and oenocyte counts especially in *B. dorsalis* larvae. The discrepancy in THC and DHC between the two host species was reflected in higher parasitoid egg encapsulation/melanisation in *B. dorsalis* for both parasitoid species. Interestingly, exposing to either parasitoid species induced the formation of the giant multinucleated hemocyte only in *B. dorsalis*. Overall, parasitism success for both parasitoids was highest on *C. cosyra*. *Psyttalia cosyrae* emerged from *C. cosyra* but not from *B. dorsalis*, whereas *D. longicaudata* exhibited the highest host immune suppression phenotypes.
Conclusion: We demonstrated that cellular immune responses are central to host-parasitoid interaction in tephritid fruit flies. Furthermore, the finding that *D. longicaudata* suppresses the host immune responses and yields more progeny compared to *P. cosyrae* suggests that this parasitoid has a greater potential as a biocontrol agent of *B. dorsalis* and *C. cosyra* in horticultural cropping systems.
Keywords: Encapsulation, Melanisation, Parasitization, Hemocytes, Immunity, *Diachasmimorpha longicaudata*, *Psytallia cosyrae*.

Poster: Natural Enemies and Biological Control

First Record of *Leptopilina victoriae* (Hymenoptera: Figitidae) associated with *Bactrocera kirki* (Froggatt) (Diptera: Tephritidae), a potential conservation biological control in Rotuma Fiji

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Background: The Biosecurity Authority of Fiji is reporting the presence of larval parasitoids of fruit fly, *Bactrocera kirki* (Froggatt) (Diptera: Tephritidae), on Rotuma Island, Fiji, located in the South Pacific Ocean, 400 miles (640km) north-northwest of Suva. we report the phylogeny of Fijian *L. victoriae* attacking *B. kirki* in early larval stages, based on COI, sequences. Reliable with molecular analysis, followed by morphological examination confirms that this is the only species of parasitoids, *L. victoriae* parasitizing *B. kirki*.
Methods: Surveys were carried out throughout the main Rotuma island from 2019 to 2020 during the fruiting season for major horticultural crops to determine host-status to fruit fly species and parasitoids in the fruits species as well as non-edible fruits from the wild areas. Fruit samples were brought to the laboratory for host rearing test where procedures include obtaining the life stages of *B. kirki* through the process of incubation.
Results: *L. victoriae* was the only common parasitoid's species found in our collections. Results from DNA barcoding of whole macerated specimens was reliably interpreted from molecular-based identifications. Partial COI sequences from *L. victoriae* showed highest sequence identity (99.54 %) to a specimen from Philippines (GenBank Accession JQ-808447) followed by a specimen from Hawaii (99.35 % with GenBank, BOLD system database Accession JQ808446).
Our samples sequences from the accessions labelled SE21-0244A – SE21-0244E except (SE21-044C) showed highest percentage identity of 99.54% to *L. victoriae* sequence from Hawaii (Kacsoh *et al*. 2012).
Conclusion: This study provides the first evidence of *L. victoriae* as recorded in Rotuma island through molecular studies. The study of the first record of *L. victoriae* biogeography of *B. kirki* parasitoids is vulnerable by the possible natural colonization and wide spread in coastal areas of Rotuma island. *L. victoriae* is associated with *B. kirki* found foraging on host *O. oppositifolia* where larval stages of fruit fly found to be feed on same hosts. Thus, *L. victoriae* parasitoids can be potentially used as a conservative biological control of *B. kirki* in Rotuma, Fiji and similar studies is also proposed in other Pacific Island countries like American Samoa, French Polynesia, Niue, Samoa, Tonga, and Wallis and Futuna where *B. kirki* populations are present and widely distributed.
Keywords: *Bactrocera kirki***,** *Leptopilina victoriae***,** COI, Conservative biological control

Poster: Natural Enemies and Biological Control

Irradiated hosts at higher doses improve parameters in fruit fly parasitoids mass rearing

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Background: Host radiation for mass rearing of fruit fly parasitoids is an essential component in the production process. Doses from 20 to 80 Gy in eggs, larvae, or pupae of different fruit fly species are applied to avoid fly emergence from unparasitized hosts, which enhances both the management and the quality of parasitoids. Higher doses can provide advantages for parasitoid rearing because the host's immune system is weakened. Irradiated larvae at higher doses were evaluated as hosts and their implications in parasitoid quality parameters. A special cellular evaluation was done to know the effects of radiation on the immunological system of the host.

Methods: Doses of 40, 80, 120, and 160 in *Anastrepha* (*ludens*, *obliqua*) and *Ceratitis capitata* larvae were applied. Irradiated *Anastrepha* larvae were exposed to Neotropical-native parasitoids *Doryctobracon crawfordi* and *Utetes anastrephae* and the South Asian- native *Diachasmimorpha longicaudata*. This exotic parasitoid was only used for *C. capitata* larvae. Weight of larva and pupa, host mortality, emergence, and sex ratio were evaluated in each treatment. Percentage of melanin in the irradiated larva was also evaluated. A special evaluation was performed with the unviable relation of parasitoid *D. crawfordi* using *A. obliqua* as host. *Anastrepha obliqua* larvae were irradiated at 160 Gy and exposed to *D. crawfordi*, larvae no irradiated and irradiated, exposed, and non-exposed were analyzed by the number of hemocytes, phagocytosis, and reactive oxygen species.

Results: No changes in weight of larvae or pupae with radiation were obtained. The doses of radiation applied also did not affect the mortality of larva after the exposition. A direct relation between the increase of radiation doses and parasitoid emergence in all species was found. Since 80 Gy, *D. crawfordi* emerged from larvae of *A. obliqua*, which is not able in unirradiated hosts. Decreased melanin was observed in *C. capitata* and *A. obliqua* larvae at higher radiation doses. The analysis of hemocytes in the larva of *A. obliqua* shown damage when they were irradiated and parasitized. The radiation reduced phagocytic activity and reactive oxygen species which have an important role in the immunological reaction against parasitism.

Conclusion: The increase of radiation in lathe larva of *A. ludens*, *A. obliqua* and *C. capitata* facilitates parasitoid development. The radiation affects the host immunological system reducing antagonist reactions against parasitoids. The use of higher doses of radiation could be very important to improve the mass rearing of fruit fly parasitoids.

Keywords: Mass rearing, Immunological reactions, Parasitism, Host availability

Poster: Natural Enemies and Biological Control

Parasitoids associated with fruit flies species (Diptera: Tephritidae) in horticulture of Madagascar

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Background: Tephritid fruit fly species were known as major pests of many horticultural crops. Among the natural enemies that regulate their populations, Hymenoptera parasitoids are commonly used in biological control programs in field situations. In the 60ies, several species belonging to the Braconidae family were introduced in Madagascar from Hawaii to control *Ceratitis malgassa* but no updated studies to evaluate their installation had been done. The purpose of this work is to provide information on the diversity, distribution, and host plant of hymenopteran parasitoid species associated with tephritid fruit flies of the island.

Material and methods: A total of 40,679 fruits (728.18 kg) from 202 plant species were sampled from 2016 to 2022. Sampling covered all the different agroecological zones of the island. Fruits were GPS located, individually weighted and incubated on river sand until pupation. Pupae were counted and individualized and parasitoids species that emerged from these samples were identified and counted.

Results: Four parasitoid species were recovered from 4.40% of the adult emerged and restricted in the East and central areas: *Fopius arisanus*, *Fopius bevisi*, *Psyttalia insignipennis* and *Psyttalia lounsburyi.* Larvae of five Tephritidae species from 12 plant species (of the 63 species identified as host of Tephritidae) were found to be parasitized by those parasitoids. Parasitism levels were variable and ranged between 0.62 to 13.91 %.

Conclusion: The most abundant parasitoid species was *F. arisanus* (90.94 %), however found in very low numbers and only in two areas of the continental island. We further discuss the implication of this species in relation to biological control of the invasive pest, *B. dorsalis*.

Keywords: *Fopius arisanus*, parasitoid species richness, Madagascar

Poster: Natural Enemies and Biological Control

Pathogenicity of *Metarhizium* and *Beauveria* isolates on preimaginal life stages of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae)

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Background: Queensland fruit fly (Qfly), *Bactrocera tryoni*, is Australia's number one fruit fly pest species. Current management practices for this highly polyphagous species primarily target the adult life stages of the fly. The use of the entomopathogenic fungi (EPF), *Metarhizium* and *Beauveria*, may provide a new biologically based method to manage Qfly populations, by targeting the soil-dwelling life stages.

Methods: The initial pathogenicity of 19 *Metarhizium* and 7 *Beauveria* isolates were screened under laboratory conditions against soil-dwelling life stages of *B. tryoni*. In replicated bioassays, isolates collected in Victoria and Queensland were screened following exposure of third instar larvae to EPF formulations of 1 x 10^7 conidia mL^{-1}, treated by mixing in vermiculite, compared to a non-inoculum control group.

Results: *Metarhizium* spp. isolates achieved an average percentage mortality of pupae with observed mycosis ranging from 27 to 92 %. The average adult emergence across local *Metarhizium* isolates ranged from 2 to 69 %. However, emerging adults from treated preimaginal stages showed a trend for a shorter life span compared to the untreated controls, with observable mycosis upon death from 13 to 77 %. By contrast, *Beauveria* isolates were less efficient than *Metarhizium* isolates, with average mortality ranging from 32% to 55%.

Conclusion: These initial results demonstrate that certain Victorian and Queensland collected *Metarhizium* isolates are highly efficacious under laboratory conditions and have potential for use as biopesticides targeting the soil-dwelling life stages of *B. tryoni*. Further work will investigate changes in the host susceptibility to infection by candidate strains of *Metarhizium* during the development of soil-dwelling life stages from late larvae to final pupal stages. Additionally, the effect of the concentration of fungal conidia (spores) applied to non-sterile soil on insect mortality will be assessed for candidate isolates, to determine the rate required for application in field conditions. Preliminary laboratory and semi-field assays conducted to date have shown the potential of selected isolates, and further research will investigate how fungal-based biopesticides may perform in an Australian orchard scenario.

Keywords: Queensland Fruit Fly, *Bactrocera tryoni*, Tephritidae, Integrated Pest Management, *Metarhizium*, *Beauveria* Entomopathogenic Fungi, Pathogenicity, Biopesticides

Poster: Natural Enemies and Biological Control

Potential of entomopathogenic fungi in the management of the invasive spotted wing Drosophila, *Drosophila suzukii* MATSUMURA

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Background: The invasive spotted wing *Drosophila suzukii* is a serious pest of soft berries, in both open field and tunnel/greenhouse production. It is native to Asia but has since invaded various parts of the world including Europe and the USA. Recently it was detected in continental Sub Sahara Africa and there are fears that its devastating effects may be amplified owing to the fragmented nature of production and poorly uncoordinated management approach. To contribute to the management of this novel pest, we screened various isolates of *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria sp*. The objective of the study was to identify potent fungal isolates for potential use as biopesticide for management of this pest.
Methods: A total of 20 fungal isolates, 13 isolates of *Metarhizium anisopliae* (ICIPE ICIPE 07, 18, 20, 30, 32, 40, 41, 60, 62, 69, 78, 84, and 387), one isolate of *Metarhizium brunneum* (Cb15-III (CECEPF) 3 isolates of *Beauveria bassiana* (ICIPE 273, 279 and 603) and 3 of *Isaria* sp (29, 679 and 682) were screened for their virulence against adult *Drosophila suzukii*. Infection of flies was done using plastic tubes whose inner walls were lined with velvet material on which 0.3 g dry conidia of each fungal isolate was evenly spread. Flies were allowed to walk on the velvet material for two minutes to allow them to pick spores before being transferred to experimental cages. The acquisition of conidia by adult flies, horizontal transmission between sexes, repeated infection by single donors, and the resultant effect on fecundity of infected flies and percent mortality were assessed
Results: Five of these isolates ICIPE 07, 18, 20, 30, and 78 were found highly potent, causing between 80-100% mortality of adult flies. There were no significant differences in the virulence of the five isolates on different ages of *D. suzukii* flies (ages 3, 5, 8 days old). However further experiments were conducted with ICIPE 18 which showed more potency. Spore uptake was high in males compared to females and more spores were present on the insect body soon after exposure to infection and decreased gradually after 24hrs post infection. Horizontal transmission between male and female with the male being the donor was not affected by age of the flies (though more conidia was transmitted by 8-day old flies) while in the opposite set up with the female being the donor, age played a significant role, with 8 day old females transmitting more conidia than 3 and 5 day old flies. Repeated infection of males when the female flies were the carriers of conidia resulted in 10% mortality in flies while the opposite scenario with males being the carriers resulted in 25% mortality of females. Infection of 3-, 5- and 8-day old females resulted in reduced fecundity in infected flies compared to the non-infected.
Conclusion: Once berries are mature, the harvesting is done every day, sometimes twice a day. This presents a challenge in managing pests in the tunnel, greenhouse or field as no synthetic pesticide has a post-harvest interval of hours or less than a day. This calls for safer alternatives which do not pose any harm to users, consumers, and the environment. Entomopathogenic fungi present such safe options and can be adopted in the berries production system.

Key words: *Metarhizium anisopliae*, Biological control, Berries, Mortality, Horizontal transmission

Poster: Natural Enemies and Biological Control

RNA interference mediated silencing of *NOA* and *V-ATPase* subunit *D* genes results in deformities and mortality in *Bactrocera dorsalis* (Hendel)

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Background*:* The fruit fly, *Bactrocera dorsalis* (Hendel) is an economically important pest and has been adjudged as one of the five most devastating fruit flies throughout the world that causes significant losses in the production of fruit and vegetable crops due to its high reproductive potential, wide host range, adaptability to climate, overlapping generations, and invasiveness. Guava is an important fruit crop of India and *B. dorsalis* has been reported to cause up to 100% fruit damage in rainy season guava. Management of *B. dorsalis* is a challenging task as they are extremely polyphagous, multivoltine, mobile, fertile, and all the life stages are unexposed except adults. The overuse of pesticides to manage this pest results in ecological backlashes and consumers' growing interest in pesticide free fruits has turned the attention of scientists towards alternative eco-friendly management strategies. The guava germplasm resilient to fruit fly is not reported in India till date.

Methods*:* We evaluated the efficiency of dsRNA expression based on *Escherichia coli* used in *B. dorsalis* management by targeting its two indispensible genes, fatty acid elongase (*NOA*) and Vacuolar-type H^+- ATPases D(*v-ATPase D*). Artificial diet having *E. coli* strain HT115 (DE3) containing Bdnoa and Bdv-ATPase D dsRNA was fed to maggots at various concentration of dsRNA, i.e., 200 µl of 200× (1×10^8 cells), 350 µl of 200× (1.75×10^8 cells), and 700 µl of 200× (3.5×10^8 cells).

Results: The feeding of dsRNA resulted in deformities along with severe mortality in treated maggots, emerged pupae, and adults. The deformity together with total mortality in *B. dorsalis* was recorded maximum as 74.66% and 64.20% in bacteria expressing dsRNAs of Bdnoa and Bdv-ATPase D, respectively at 700 µl as compared to 350 and 200 µl treatments. Severe deformities were also observed such as underdeveloped abdomen, curled wings, absence of wings and legs, inadequate eclosion, melanisation, along with suppressed target genes expression in *B. dorsalis*.

Conclusion: Our investigation provides a basis for the practicality of utilization of bacteria expressing dsRNA through feeding to *B. dorsalis* developmental stages to silence two vital genes (*NOA* and *V-ATPase* subunit*D*) which subsequently produced severe deformities and mortality in *B. dorsalis*. In this perspective, we intend to prepare a ihpRNA construct (RNAi construct) marker-free by using same *B. dorsalis NOA* gene and also standardize *in planta* transformation using floral dip

method in ‘Allahabad Safeda’, a popular cultivar of guava in India to produce RNAi-based fruit fly resistant guava plants could be realize in coming years.

Key Words: *Bactrocera dorsalis*, RNA interference, dsRNA, deformities, mortality

Poster: Natural Enemies and Biological Control

The potential of entomopathogenic nematodes as biocontrol agents against Queensland fruit fly

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Background:
Entomopathogenic nematodes (EPNs) from the families of Heterorhabditidae and Steinernematidae are important biocontrol agents of insect pests with soil-inhabiting stages. They have been used as a safe alternative to chemical pesticides. However, the potential of EPNs to control Queensland fruit fly (*Bactrocera tryoni*) is largely unknown. Furthermore, their control efficacy depends largely on their ability to survive long enough till an insect host becomes available.

Methods:
We tested 17 Australian isolates belonging to five EPN species to see if they can survive without hosts and remain virulent for several weeks. We also tested whether fruit fly can be infected by providing adults with an aqueous yeast solution containing EPNs. Furthermore, we assessed the flight ability of adults after EPN infection. Finally, a semi-field assay is ongoing to test the virulence and persistence of the high performing four EPN isolates against different stages of *B. tryoni.*

Results:
We found that after 1, 2 and 3 weeks without a host, all EPN isolates still infected and killed *B. tryoni* larvae, while after 7 and 14 days without host, 15 and 2 isolates, respectively, still infected and killed *B. tryoni* pupae. The adult feeding experiment resulted in 48 (± 6) % to 75 (± 6) % mortality due to EPN infection after 7 days. Moreover, up to 21 ± 3 %, 30 ± 5 % and 34 ± 4 % of EPN infected flies were able to fly one day, two and three days after EPN infection, respectively. Finally, semi-field assay has resulted in high levels of EPN infections in larvae entering the soil, pupae and emerging flies with higher levels of fruit fly mortality than seen in laboratory assays.

Conclusions:
In conclusion, we found EPNs can infect fruit fly across several developmental stages and cause high levels of mortality. Furthermore, they persisted in the absence of insect hosts for several weeks. Yeast solution containing EPNs can be used in an attract and kill strategy targeting adult *B. tryoni*. Finally, the dispersal of EPN by infected fruit flies could help the dissemination of EPNs in the landscape and further aid in the wider control of fruit fly by EPNs.

Keywords:
Queensland fruit fly, *Bactrocera tryoni*, biological control, entomopathogenic nematodes

Poster: Natural Enemies and Biological Control

Unravelling chemical cues used by *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) during host search.

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Background: *Diachasmimorpha longicaudata* is one of the most commonly used biocontrol agents against Tephritidae fruit fly pests. Females parasitize their hosts during the larval stage when they are still feeding inside the fruit. In order to locate these larvae, females use semiochemicals from the microhabitat. Using a stationary olfactometer (no air flux), previous studies have shown that oranges infested with *Ceratitis capitata* (Diptera: Tephritidae) larvae, infested oranges from which all larvae had exited, rotten (non-infested) oranges, and oranges infected by the fungus *Penicillum spp.* were preferred by female parasitoids when offered in dual choice tests with healthy oranges (control). Our aims were to confirm attraction to these sources using a Y-tube olfactometer (air flux) and to identify the chemical compounds responsible for the attraction of *D. longicaudata*.

Methods: Previous experiments were repeated in a Y-tube olfactometer using a vacuum pump. Each parasitoid was tested on its first choice when offering a treated (same as above) vs. a healthy orange. Then, the volatiles of these oranges were collected using HaySep-Q traps for 4h and desorbed with 300 µl dichloromethane. Samples were used in GC-EAD experiments to determine which of the compounds were antennaly active. This was followed by GC-MS analyses to identify those compounds shared by the attractive oranges but were absent in healthy ones. Using EAD, a dose-response curve for each compound was obtained.

Results: As in stationary olfactometers, *D. longicaudata* females oriented preferentially towards treated oranges in the Y-tube olfactometer. GC-EAD showed several antennaly active compounds but only seven of them belonged exclusively to the treated and not to the healthy oranges. According to GC-MS (NIST library and Kovats index) these compounds were identified as: octanal, limonene, acetophenone, linalool, nonanal, decanal and eugenol. EAD responses to some of these compounds showed to be highly dependent on the applied concentration but others were independent to this factor.

Conclusion: *Diachasmimorpha longicaudata* females were attracted to volatile compounds emitted by different sources associated to host presence. At a peripheral level of the nervous system, the compounds triggered different patterns of responses. This suggests different processing pathways at higher levels of the nervous system, thus behavioural experiments are needed. Bioassays in a wind tunnel are planned to search for a bait that could serve to monitor parasitoid populations and to pull them into fruit crops under biological control programs.

Keywords: Chemical ecology, Semiochemicals, Parasitoid, Tephritidae, Biological control

Poster: Other Control Methods or New Developments

A Novel Real-time Sensing Trap to Detect Oriental Fruit Fly (*Bactrocera dorsalis)*

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Background: We proposed that a sensor measuring capacitance change of the electrodes to detect the oriental fruit fly pass by the trap. This sensor is more sensitive than the infrared photodiode and can save more energy in the same working conditions. Use MCU to report in real time through 4G LTE, to build a battery-powered IoT system to monitor the amount of oriental fruit flies.
Methods: In this study, capacitive sensing electrodes are used to measure the signal characteristics of fruit flies passing through the sensor, and the operating current consumption of the sensor is compared with infrared photodiode in the same working conditions.
Results: In comparison with infrared photodiodes, capacitive sensors require only 10% of the working current. The results show that the current consumption of the maximum capacitive sensor consumes only 155.53uA during continuous operation, and the detection sensitivity is 0.01nF, accuracy of the number of detecting insects is 92%.
Conclusion: Through the MCU program to organize different work situations, such as sensing, sleep, schedule data-transmission cycles to reach the best available battery time. Thus, adding a solar panel to charge the battery will enable the entire system to work in the long term.

Keywords: Field internet of things; capacitive insect sensor; Smart agriculture; Pest monitoring

Poster: Other Control Methods or New Developments

Essential oils as insecticides and repellents of Queensland fruit fly

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Background: Tephritid fruit flies are amongst the world's most economically damaging insect pests, impacting the quality and trade of fleshy fruits and vegetables in tropical and subtropical regions. High residual broad-spectrum synthetic pesticides have been used to control fruit flies for many years. However, such pesticides can pose toxicological threats to humans and has detrimental effects on the environment and non-target organisms. These concerns have led to the development of other options, including essential oils (EOs) as softer insecticides. This study aims to investigate essential oils as botanical insecticides and repellents for the Queensland fruit fly (Qfly), *Bactrocera tryoni*.

Methods: The toxicities of EOs were evaluated against *Bactrocera tryoni* using contact and fumigation assays. The repellent activities of EOs were evaluated by oviposition deterrence and four-arm olfactometer assays. Composition of each EO was analyzed by gas chromatography-mass spectrometry (GC-MS). Gas chromatography-electroantennogram detection (GC-EAD) was used to identify electrophysiologically active compound(s) that might be responsible for repellent activities. Microcapsules of EOs that induced oviposition deterrence were produced by *in situ* polymerisation and were tested for effectiveness at reducing oviposition on fruit in enclosures.

Results: Java citronella, citronella grass, lemon-scented tea, aniseed, yarrow, pennyroyal, thyme white, and chamomile EOs showed high toxicities to both sexes (45 – 76%) in 24 h after topical application in 10% solutions. Aniseed, eucalyptus, yarrow, pennyroyal, thyme white, tea tree, peppermint, garlic, dill seed, and basil exhibited excellent fumigation toxicity (100% mortality) in 24 h when 5 µL of each EO was used, while java citronella, citronella grass, lemon-scented tea and cumin seed exhibited moderate toxicities (50 – 95% mortality). EOs also varied in repellence, and detailed analysis is ongoing.

Conclusion: The current investigation with Qfly found that java citronella, citronella grass, lemon-scented tea, and chamomile EOs are candidates for topical toxicants, while aniseed, eucalyptus, thyme white, peppermint, garlic, dill seed, and basil EOs are candidates for fumigants. Chemistry of EO, oviposition deterrent activities, microencapsulation, and bioassays will be discussed.

Keywords: *Bactrocera tryoni*, botanicals, toxicants, fumigants, oviposition deterrents

Poster: Other Control Methods or New Developments

Pilot Study on Melon fly Suppression in Seychelles

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Background: Melon fly, *Zeugodacus curcubitae* was introduced to Seychelles in 1999 and has become a major pest on cucurbits in the main Islands of the Archipelago. After the introduction, the Seychelles government initiated a campaign to eradicate melon fly in the country. However, due to technical and economic reasons the objective was changed to suppression. With this objective, a suppression pilot study was conducted for 3 months (cucumber crop cycle) on Mahe Island.
Method: The study area was of about 8 km^2 and 119 farmers were concerned. The following suppression methods were applied: (1) sanitation; (2) Male Annihilation Technique (MAT) blocks with Cuelure (CUE); (3) bait spray with GF120 (spinosad); and (4) mass-trapping with GF120. To evaluate the impact of the suppression, a trapping network of 41 tephri traps with CUE as attractant was installed and fruit sampling conducted pre- and during the pilot trial.
Results: Adult populations captured in CUE traps decreased from about 6 FTD (fly per trap per day) to <1 FTD and fruit infestation reduced from about 90% at commencement to 20% during the trial. Farmers' incomes increased due to more marketable cucurbits.
Conclusions: These promising results showed the importance of integrating several fruit fly management methods and the advantages of an area-wide approach to population control rather than the field by field approach. A follow-up study to combine the above suppression methods with the Sterile Insect Technique is planned for initiation as soon as sterile flies are available.

Keywords: *Zeugodacus curcubitae*, sanitation, male annihilation technique, bait sprays, bait stations, adult surveillance, larval surveillance, fruit infestation.

Poster: Other Control Methods or New Developments

Susceptibility of fruit fly, *Bactorcera* (*Zeugodacus*) *zahadi* Mahmood to native strains of entomopathogenic nematodes from North Western Himalayan region

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The fruit flies from genus *Bactorcera* are considered as a major pest throughout India and their incidence has been serious in Himachal Pradesh. The fruit fly, *Bactorcera* (*Zeugodacus*) *zahadi* is one of the major insect pests of cucurbit vegetables under mid-hills of Himachal Pradesh (Nauni). The present study aimed to evaluate the two indigenous Entomopathogenic Nematodes (EPNs) strains, *Heterorhabditis bacteriophora* HR2 and *Steinernema feltiae* HR1, against distinct developmental stages of fruit fly, *i.e.* larvae and pupae. Native EPNs strains were isolated and laboratory bioassay studies were done to determine their infectivity. The maggots and pupae of *B. tau* were exposed to EPNs in Petri plates lined with Whatman No.1 filter paper and their susceptibility to nematode infection was determined at various concentrations of 10, 20, 40, 80, and 160 IJs/cm2. The 10 larvae and pupae of the test insect were added and allowed to burrow into the substrate within 1 hour of IJs treatment. Both EPN species induced varying amounts of mortality in petri plates containing 10 larval insects, with maximums of 68.66 percent and 54.66 percent caused by *H. bacteriophora* and *S. feltiae*, respectively after seven days of exposure at 160 IJs /cm^2. After seven days of exposure time in plastic jars, various levels of mortality caused by *H. bacteriophora* and *S. feltiae* was documented, with maximums of 53.33 percent and 50.00 per cent caused by *H. bacteriophora* and caused by *S. feltiae*, respectively. Based on its higher virulence and better ability to penetrate the larval/pupal tissue, the results indicated that *H. bacteriophora* was found to be the most effective species and may have potential as a biocontrol agent of *B. zahadi*. This study revealed that these two indigenous strains of Entomopathogenic nematodes (HR1 and HR2) showed the potential for control of distinct developing stages of fruit fly under laboratory conditions *i.e.* caused significant mortality of last instar maggot and pupal stages. Further field testing can be performed for incorporating these indigenous strains of EPNs in an integrated pest management programme for fruit fly management under the mid hills of Himachal Pradesh, India.

Keywords: *Bacterocera zahadi* Mahmood, Entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Steinernema feltiae*, Biocontrol

Poster: Area-wide Integrated Pest Management and Action Programs

Alternate hosts associated with Oriental Fruit Fly *Bactrocera dorsalis* Hendel, the main pest of Indonesian Mango var. Gedong Gincu.

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Background: Fruit flies are economically important pests of tropical fruits including mango (*Mangifera indica* L.). An Australian Centre for International Agriculture Research (ACIAR) funded project has implemented an Area-Wide Management (AWM) program to control fruit flies in smallholder mango orchards at Krasak village, West Java. The neighbouring village of Pawidean was used as a control site. Several alternate hosts of fruit fly such as guava, starfruit, water apple and others are commonly found around mango orchards in Indonesia. This study investigated the potential of alternate hosts to contribute to the fruit fly populations in these mango orchards.

Methods: Mango, guava, star fruit and water apple fruits were sampled during May 2021 – May 2022 at 2 locations (Krasak and Pawidean) in Indramayu, West Java, Indonesia to assess the level of infestation by fruit flies. Mango was sampled from within the AWM zone, and guava, starfruit, and water apple were sampled from nearby villages outside of the AWM zone. A total of 800 mango, 401 guava, 519 starfruit, and 975 water apple fruits were sampled from Krasak, while 800 mango 364 guava, 563 starfruit, and 781 water apple fruits were collected from Pawidean. Such samples of alternate host fruits were collected monthly and the fruits were set up in rearing containers. The adult flies that emerged from the different host fruits were then counted and identified to species level.

Results: The study showed that infestation rate of "Gedong Gincu" mango sampled from within the AWM zone at Krasak was less than 1%, whereas at the control site in Pawidean 25% of the mangoes were infested. The predominant species of fruit fly reared from mango was the oriental fruit fly *B. dorsalis*. Fruit fly infestation rates in guava, starfruit, and water apple sampled from bordering villages outside the AWM area at Krasak were 45%, 55% and 29%, while at Pawidean infestation levels were 44%, 48% and 33% respectively. Beside *B. dorsalis,* the alternate hosts were also infested by *Bactrocera carambolae* and *Bactrocera albistrigata*. No *B. albistrigata* were reared form mango.

Conclusion: The extremely low rate of infestation which is less than 1% for mango produced within the AWM zone at Krasak compared to 25% in the control site at Pawidean illustrates that the AWM method is highly effective in managing fruit flies in mango farms surrounded by high endemic populations of fruit flies. Alternate host fruits also provide an abundance of fruits for fruit flies to breed in and sanitation or collection and destruction of unwanted host fruit is an important component of the AWM program.

Keywords: *Bactrocera dorsalis*, Gedong Gincu, infestation rate.

Poster: Area-wide Integrated Pest Management and Action Programs

Area-wide Control Programs for Chinese Citrus Fly (*Bactrocera minax*（Enderlein)) in Eight Citrus Growing Provinces in China and Nepal using GREAT® Fruit Fly Bait

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Background: Chinese Citrus Fly (CCF) (*Bactrocera minax*（Enderlein)) is one of the most destructive pests of Citrus crops. Found in China, Nepal and parts of India, it is included in the list of international quarantine pests in many countries. It causes extensive fruit damage rate ranging from 25% in most infested areas to as high as 100% in severe cases.
From 2012-2021, a number of area-wide control programs (AWCPs) have been implemented in citrus growing areas in 8 provinces in China - Hunan, Chongqing, Hubei, Sichuan, Guizhou, Yunnan, Shaanxi and Jiangxi. The data of infestations of CCF and control effect of AWCP using Great® Fruit Fly Bait (GFFB) over 10 consecutive years were collected and analysed. In addition, an AWCP was conducted in 40 ha of sweet orange orchards at Sindhuli, Nepal in 2018.
Methods: Conventional control and GFFB mass baiting treatments were set up in each test area. Fruit fly population was monitored using Ecoman® fruit fly trap with GFFB from late April to mid-May. Application of GFFB started 7 days after CCF early emergence time was determined. One litre GFFB was spot sprayed at 60-70 spots per acre every 7 days, with a total of 4 rounds of application for Ponkan, 6 rounds for Orange & Pomelo and 10-12 times for sweet orange in Nepal.
Results: A total of 237,000 hectares were applied with GFFB from 2012-2021. The average fruit damage rate dropped from around 20% to about 0.5%. In 2021, the average fruit damage rate in GFFB treated zone was 0.5%, which was significantly better than conventional control zone of 6.33% ($P<0.05$). With an average yield of 30 tons/ha of citrus fruits at an unit price of 0.5 USD/kg, GFFB-based AWCP has helped increase the growers' income by an estimated 81.5 million USD over ten years as a result of increased yield and saved labor costs (18 million USD).
Conclusions: Past 10 years data of CCF control shows that the application of GFFB for 3 consecutive years can reduce the average fruit damage rate from >20% to 1%. It further went down to 0.5% when applied for 5 consecutive years.
GFFB is simple to use, affordable, highly effective and convenient for area-wide applications. The spot application method reduces the overuse of harmful pesticides, labor costs and impact on the environment. Therefore, GFFB has an important economic and ecological significance in the control of Chinese Citrus fly in China.

Keywords: Great® Fruit Fly Bait (GFFB), *Bactrocera minax*, Fruit Damage Rate Decrease, Income Increase

Poster: Area-wide Integrated Pest Management and Action Programs

Area-Wide Management Approaches for Fruit Flies in Indonesia

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Background: Indonesia is the fourth largest producer of mangoes in the world. However, production and market access are restricted by two species of fruit fly, *Bactrocera dorsalis* and *B. carambolae,* which cause significant damage to mango in Indonesia. A coordinated area-wide systems approach incorporating **male annihilation, protein bait spraying, crop hygiene and population monitoring, together with farmer training** was implemented in two production areas in West Java.

Methods: The trial sites were located at Krasak (40ha, district of Indramayu) and Sedong Lor (25ha, district of Cirebon). The orchards were surrounded by village dwellings and rice fields. The standard insecticide treated orchards used as control sites were located at Putat (45ha) and Pawidean (53ha). The male annihilation technique (MAT) blocks were **4:1 methyl eugenol and fipronil toxicant in a** 5x5cm fibreboard block. The blocks contained approximately 12 ml of product each and were nailed onto trees at 50m intervals (about 6 blocks/ha) and placed in the orchard as well as in the village. New blocks were added every 2 months. Protein baits based on a brewery yeast by-product and containing the toxicant abamectin were applied weekly and commenced after the oldest fruits were aged 4 weeks after fruit set and continued until all fruits were harvested. Four spot sprays of 25ml each were applied per tree. Fruit was visually observed at the packhouse and samples of 100 fruit/sampling were kept and incubated for 3 days and then dissected to confirm presence or absence of eggs and larvae. Fruits were sampled monthly during the season.

Results: At the start of the AWM program in August 2019, the number of flies/trap/day (FTD) reached a high of 12.27 in Krasak and 14.24 in Sedong Lor. It then took about 6 months for the populations to reduce and stabilise to below the suppression level of <1 Fly/Trap/Day (FTD). Populations have been maintained below the suppression level of <1 FTD from the 6 month after initiation of the program until the present. In comparison, the FTD value in the control sites recorded a high of 468 in Putat and 863 in Pawidean. In 2021 the overall damage was 0.2% at Krasak and Sedong Lor. Infestation levels at insecticide treated sites, trader warehouses and local markets ranged between 6-31%.

Conclusion: Farmers are self-sufficient and able to maintain the AWM program under a coordinated approach. Expansion is planned for areas in East and West Java. The area-wide systems approach is suitable for orchards as small as 25ha and for other commodities.

Keywords: Male Annihilation, Protein baiting, Area Wide Management

Poster: Area-wide Integrated Pest Management and Action Programs

Area-wide management of Queensland fruit flies in Victoria's Goulburn Murray Valley by a combined government, industry, community and science approach

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Background:Goulburn Murray Valley (GMV) is a horticultural region in Victoria mainly growing stone and pome fruit. Queensland fruit fly established in the region in 2013. In 2017 the Goulburn Murray Valley fruit fly Area Wide Management program was established to build community awareness and ownership of Queensland fruit fly management in the region and to suppress fruit fly populations. A key to achieving these aims is the involvement of a range of regional stakeholders including community groups, local and state governments and local horticulture industry associations.

Methods: The GMV Governance Group, representing Agriculture Victoria, local councils, horticultural industries/grower organisations, community groups and scientists developed area-wide management (AWM) action/implementation plans. The govenance group members and the coordinator made a case for participation in delivery of the plan to a range of stakeholders through meetings and presentations to outline how their contribution could assist the regional to improve the management of Queensland fruit flies. Queensland fruit flies were monitored weekly during the fruit growing season and fortnightly in the winter.

Results: This initiative resulted in the GMV fruit fly program achieving ongoing volunteer support and ownership which led to broad community engagement across the entire region.

Activities by approximately 3000 volunteers included - assisting with the installation of 501 road signs to build awareness of fruit flies, workshops for producers and the community, field days and a school-based education program which in total reached 16,954 people and distributed 53,996 information packs. State and local governments provided points of contact for the community linking them to fruit fly information and voluntary tree removal programs. Tree removals by GMV local governments removed 22,285 trees from home gardens and public land and 81,662 trees from unmanaged orchards. Industry groups participated in media campaigns to encourage the community to act to manage fruit fly. This complemented the other work by the program such as fruit fly monitoring, host plant removals, monthly fruit fly situation updates, mass-media awareness, education and support for the SIT pilot in the GMV.

The program achieved broad-based community engagement about fruit flies which would not have been possible without the involvement of all regional stakeholders. There has been a reduction in the fruit fly numbers over the four years of the program compared to the previous four years.

Conclusion: AWM programs require the support of diverse stakeholders to engage with the community and promote change. Engagement is likely to be more successful with a whole-of-

community approach based on the active involvement of government, industry, community and the use of science-based decision making.

Keywords: Queensland fruit fly, Area-wide management, whole-of-community approach

Poster: Area-wide Integrated Pest Management and Action Programs

Developing the Marking Procedures for Studying the Migration of *Bactrocera dorsalis*

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Background: Understanding insect migration is a key component for the success of sustainable Area-Wide Management (AWM). *Bactrocera dorsalis* is a key pest of many agriculturally important crops, and it has been the subject of pest management and quarantine issues. AWM for this insect has been implemented through an Australian Centre for International Agricultural Research (ACIAR) funded project in Java, Indonesia, and information on the migration patterns of adult flies are needed to mitigate the movement of flies from outside into the AWM zones. Mark-release-recapture (MRR) is one of the best methods to study adult fly migration, and marking the fruit fly pupae with fluorescent powder is one of the common marking methods used to conduct MRR studies. This paper describes the development of a reliable marking procedure which has not been well established for *B. dorsalis.*

Methods: Studies on marking with fluorescent dye for *B. dorsalis* and subsequent detection of marked flies was conducted between 2020-2022. This was done in 3 stages to achieve a reliable marking protocol. In the first stage, the pupae were coated directly with fluorescent powder at a concentration of 1.5 g/L pupae (FAO standard). The marked pupae were then mixed with the unmarked flies in ratios of 1:10, 1:100, and 10:100 and observed with a dissecting microscope under the UV light at 10, 12, and 14 days after emergence from the pupae. An improvement to the marking and observation technique was made in the second stage. The pupae were marked by using a cosmetic fine brush to provide even coverage of the fluorescent dye. Before observation, the heads of adult flies were soaked in alcohol and water. The last stage was conducted to compare the efficiency of marking between direct pupal coating and dyed vermiculite as the media for adult emergence.

Results: The first stage of development resulted in 67-100% recovery of the marked flies. After improving the marking and observation techniques, the visibility and recovery of marked flies increased greatly with a range of 99.3-100%. The percentage of live flies up to seven days after emergence from dyed vermiculite method was significantly lower than that from the direct coating method.

Conclusion: The improved marking and observation techniques were more convenient, created less mortality and increased the accuracy in observing the marked *B. dorsalis* flies

Keywords: Oriental fruit fly, area-wide management, mark-release-recapture.

Poster: Area-wide Integrated Pest Management and Action Programs

Does Pupal size matter for field dispersal and prevalence of laboratory-reared sterile Queensland fruit fly?

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Background: The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) or 'Q-fly', is the most economically damaging horticultural insect pest in Australia, attacking a vast variety of fruit and vegetable crops. The sterile insect technique (SIT) provides an environmentally friendly tool for effective regional management of insect pests, including Q-fly. The success of fruit fly SIT, including Q-fly, depends on dispersal, survival, nutrition, and ability to attract and mate with wild females. High abundance of sterile males in the field is essential for SIT, and this is driven by both (1) production and release of large numbers of competitive sterile males and (2) survivorship and health of released insects. Size is often discussed as a quality metric, but there is little field data available in relation to how size affects post-release performance.
Methods: A pupal sorting machine was used to separate pupae into three different size classes (small, medium, and large) by diameter, after which each size class was marked with a different dye. On day 5 after emerging, adult flies that emerged from small, medium, and large pupae were released. Before releasing marked flies, 12 cuelure traps were set up co-axially. We analyzed the recaptures of Q-fly at different distances from the release point. Cuelure targets mature males and so numbers reflect both maturation and survivorship. Trials were replicated five times.
Results: Probability of recapture significantly differed for males that emerged from small, medium, and large pupae. The overall probability of recapture was significantly lower for flies that emerged from large pupae than for flies that emerged from small and medium-size pupae. Probability of recapture declined with distance from the release point, regardless of the size of the pupae that the flies emerged from.
Conclusions: The size-dependent recapture of sterile Q-fly has implications for population dynamics and pest control strategies. Our findings suggest that the operational SIT of Q-fly might be more successful by targeting production of pupae with size that produces the strongest mature male recapture results in the field although consideration also needs to be given to relationship between size and sexual performance.

Keywords: Horticultural pest, Sterile field release, Dispersal, Prevalence

Poster: Area-wide Integrated Pest Management and Action Programs

Effect Of Community Participation On Suppression Of *Bactrocera Dorsalis* (Hendel) ((Diptera: Tephritidae) In Mango Orchards In Zanzibar

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Mango production is the source of income and livelihood for small scale farmers in Zanzibar. The oriental invasive fruit fly (*Bactrocera dorsalis*) is a major threat and cause devastating loss in fruit and vegetable production. Gulf and European countries have imposed strict trade restrictions on mango consignment which has negative impact on export trader, mango stakeholders, income and food security of many rural households. Farmer's community were empowered by the Ministry of Agriculture on suppression on fruit fly by Integrated Pest Management (IPM) program by creating public awareness in rural areas, conducting training at regional and farmers level, Provision of ±1,500 trap baits of Male Annihilation Technique (MAT) and food bait to small scale farmers. Other practices employed were orchard sanitation, cover sprays and fruit sampling. The program was executed in 10 villages in South Region from 2017 - 2018. By using baits *B. dorsalis* population have been reduced from 5.5 fruit fly per trap per day (FTD) to 0.5 (FTD) during the major mango season. During the lower mango season was 2.6 (FTD) to 0.7 (FTD). Level of infestation by fruit sampling has been reduced from 90- 25%.The result suggest that the community can contribute on implementation of fruit fly suppression program and it can to be effective. For this program to be sustainable there is a need to implement these practices on Area Wide IPM program with farming community groups in other regions of Zanzibar.

Keywords: Community, Area Wide IPM, public awareness, suppression

Poster: Area-wide Integrated Pest Management and Action Programs

Effect of semio-chemicals (Methyl Eugenol -ME) on male reproductive organs, mating competitiveness and reduce response to ME baited traps of *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae)

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Background: Males of many *Bactrocera* species under the family Tephritidae are strongly attracted to methyl eugenol (ME), a naturally occurring compound known as semio-chemicals. Different semio-chemicals/male lures have successfully been implemented in the male annihilation technique (MAT), or in-corporated in sterile insect technique (SIT) programmes as pre-release treatments. Semio-chemicals also have a lasting effect of reduced responsiveness to male lures, and might provide a basis for development of simultaneous deployment of MAT and SIT. In the present study efforts were made to determine the impact of ME incorporated diets and ME aromatherapy as pre-release treatments on reproductive organs, improvement of sexual performance as well as reduce response to traps used in MAT of *Bactrocera dorsalis* (Hendel).
Methods: We incorporated ME into *B. dorsalis* adult diet of sugar only or sugar mixed with yeast hydrolysate after adult emergence. The size of male reproductive organs (testes and apodeme) was recorded on day eight fed on above mentioned diets. The mating performance of ME aroma-treated fertile and sterile *B. dorsalis* males was observed and recorded under laboratory condition and semi-field cages. The potential of pre-release ME aromatherapy as a means of reducing response of fertile and sterile male *B. dorsalis* to ME lure was determined under semi-field cages.
Results: ME incorporated sugar diets showed significant influence on the testes and apodeme size of eight days old *B. dorsalis* males compared to those fed only sugar diet. ME-aroma-treated fertile and sterile *B. dorsalis* males showed enhanced mating success over untreated males in single mating experiments under laboratory condition. Significantly higher mating pairs (66 %) was recorded for ME aroma-treated sterile male *B. dorsalis* over untreated males (34%). The mating latency and mating duration of the above mentioned mating tests also differed significantly. A similar trend of mating performance was recorded in semi-field cage trials. *B. dorsalis* recaptured in ME-lure baited traps after three days of ME post treatment (DPT) differed significantly under small semi-field cage trials. The percentages of ME treated fertile and sterile *B. dorsalis* males recaptured were significantly less than those of corresponding control.
Conclusions: Experimental results confirm the influence of ME as prerelease supplement while incorporated with adult diets as well as aromatherapy on reproductive organs and mating successes of male *B. dorsalis*. The reducing capture of ME aroma treated males *B. dorsalis* in ME baited traps indicated the potential of ME on simultaneous application of SIT and MAT that would be a benefit of area-wide management programmes of *B. dorsalis.*

Key words: Oriental Fruit fly, Semio-chemicals, Adult diets, Mating success, Reduce response

Poster: Area-wide Integrated Pest Management and Action Programs

Essential elements for MedFly AW-IPM success as a strategy for friendly pest control

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Background: MedFly (Ceratitis capitata Wiedemann) is a key pest in Israeli fruit orchards, and its management is based on sustainable methods within area wide integrated pest management (AW-IPM interface). In Israel, about 20,000 hectares of commercial fruit orchards are being hosts of MedFly. The Israeli agriculture is characterized by a number of factors that affect pest management in general, and MedFly in particular: A large varieties of MedFly hosts, which are available all year round; proximity to natural wild vegetation adds a variety of hosts; a total ban of strong, unfriendly pesticides; increasing frequency of extreme climatic events and conditions that encourage MedFly population development; the human factor (growers) that adds a complexity to the implementation process.

Methods & results: The MedFly management strategy developed in Israel was formulated into an applied protocol, including the following elements:

- Community based MedFly AW-IPM, combining a top-down regional administration, with activities being carried out by the farmers themselves (bottom-up).
- Regional cooperation between over 1,200 growers, managed by a professional team and cooperation between all farmers-relevant organizations (XBC-Cross boundaries Collaborations).
- MedFly control based on mass trapping, ongoing monitoring and spraying with friendly materials only by actual necessity
- Use of a variety of mass trapping devices: Top Trap (SEDEC, Rimi Ltd.), Cera Trap (Bioiberica, Gadotagro Ltd.), Luretect (Ronpal, Adama-Agan), NMF (Bio-Bee) and Freedome (Biofeed) and ongoing professional examination of their effectiveness. Device type was selected according to the host and the environmental conditions, emphasizing the usage of same device type throughout a specific large area along the year.
- Precise MedFly control in sub-plots hot spots, including the use of drones.
- Improving MedFly adult population monitoring by developing automatic monitoring traps.

In addition, the protocol was supported by a study of common horticultural treatments (for fruit quality improving) effecting the attraction and rejection of MedFly.

Conclusions and Insights: An analysis of the impact of the regional MedFly management initiative, including an examination according to the SWOT model showed that the protocol described above led to a significant reduction in the use of toxic pesticides (also in the EIQ index), while no additional fruit damage was observed. The regional management team provides a comprehensive professional support framework of plant protection to growers, which guides them in the transition to a comprehensive culture of sustainable and environmentally friendly agriculture.

Key Words: Mediterranean fruit fly, MedFly, Ceratitis capitate Wiedeman, IPM, AWM, Mass trapping, attract and kill, precision pest control, action program, monitoring, automatic monitoring traps

Poster: Area-wide Integrated Pest Management and Action Programs

Exploration of an alternative material for ant repellants used in surveillance traps for invasive fruit flies

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Background: Surveillance trapping is indispensable measure for the establishment and maintenance of a fruit fly-pest free area. However, predation on trapped fruit flies hinders the effectiveness of the surveillance traps. Ants, in particular, are the most devastative predators because they can carry away the entire insect body, the evidence of the pest's presence. In this study, we evaluated the effectiveness of a lubricant grease (Grease), a cost-effective and readily available products, as an alternative to a popular ant repellent, Tangle (Tree Tanglefoot Insect Barrier®).

Methods: We prepared a Steiner-type trap attached Tangle or Grease as ant repellants and Control without any materials. Ten traps for each treatment were set by hanging from trees. Twenty frozen-dead melon flies, *Zeugodacus cucurbitae*, were set in each trap and collected them two weeks later to evaluate predatory damage. The number of flies disappeared and damaged by predatory attacks were counted. The predators observed at the time of collection were also recorded. This procedure was repeated 3 times.

Results: The number of traps with predatory attacks were not significantly different among treatments with and without ant repellants. However, when disappearance damage occurred in Control, almost all flies (19.0±2.1: mean±SD) disappeared from the traps. On the contrary, disappearance damage on the traps with Tangle and Grease were limited (Tangle: 3.3±2.9 and Grease: 1.0±0.0, respectively). Regarding disappearance damage rate per fly, traps with Grease were slightly but significantly less damaging than traps with Tangle. Although we observed several predators (ants, spiders, cockroaches and larvae of Erotylidae beetle), intruding the traps for all the treatments, ants were observed only in Control traps.

Conclusion: Easily available material “Grease”, an industrial machinery lubricant, is an effective alternative of ant repellant for using fruit fly surveillance trap.

Keywords: ant repellent, invasion survey, lubricant grease, predation, Steiner trap, surveillance traps

Poster: Area-wide Integrated Pest Management and Action Programs

Extension Activities of Area-Wide Management Approaches for Fruit Flies in Indonesia

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Background: Indonesia is ranked fourth in global mango production with about 2.86 million metric tons produced in 2021. However, infestation by two species of tephritid fruit flies, namely *Bactrocera dorsalis* and *B. carambolae*, severely impact yields and fruit quality resulting in reduced incomes for farmers. Fruit fly management in Indonesia is presently based on insecticidal cover sprays and ad hoc male fruit fly trapping, both of which provide inadequate control. The Australian Centre for International Agricultural Research has consequently funded Area-wide fruit fly management research in West Java. The Area-Wide Management (AWM) program is based on a combination of male annihilation (MA), protein baiting, sanitation, adult fly population monitoring and farmer training and coordination, and has successfully reduced and maintained fly numbers at less than 1 fly/trap/day in mango orchards. Based on the success of this AWM program, the Directorate General of Horticulture (DGH) together with the Indonesian Tropical Fruit Research Institute (ITFRI) have been extending the results of the research and promoted the establishment of such programs to non-participating districts throughout Indonesia.

Methods: The AWM program has been promoted to other districts through a series of online webinars to more than 1000 participants. Following the easing of Covid-19 restrictions, participant capped face to face workshops were delivered to 80 attendees at each location in Majalengka, Sumedang, Pasuruan and Situbondo. Initial webinars and seminars targeted extension officers and pest observers from various regions throughout Indonesia. This program is based on the “train the trainer” concept.

Results: Due to success of the ACIAR funded AWM program in West Java where populations have been reduced to <1 flies/trap/day and infestations < 1%, regional pilot AWM programs have been initiated. Programs have been implemented by the local governments at Sumedang (125ha), Indramayu (100ha), Majalengka (87ha) and Cirebon (88ha). To date, monitoring, MA and sanitation in a coordinated approach have been implemented. Protein baiting will commence after fruit set. Further programs, initiated by DGH will be implemented at Kertajati, Majalengka, West Java (33ha) and Gresik, East Java (20ha). Both sites are being negotiated with the local governments.

Conclusion: Fruit fly populations can be reduced by area-wide management in fruit production sites. Collaboration among stakeholder is a must. Dissemination both online and physical has increased grower and local government interest in initiating fruit fly AWM programs in various regions in

Indonesia. Presentations by the project participating farmer has increased the impact of the presentations. The program is suitable for most fruit fly susceptible commodities.

Keywords: Male Annihilation, Protein baiting, Area Wide Management

Poster: Area-wide Integrated Pest Management and Action Programs

Fruit fly infestation levels on mango var. Gedong gincu sampled from several supply chains in West Java, Indonesia

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Background: Fruit fly is the most devastating and economically important pest of mango (*Mangifera indica* L.) in Indonesia. Control at the farm level is highly variable and is based largely on ad hoc cover sprays of insecticides. An Australian Centre for International Agricultural Research (ACIAR) project initiated in 2018 has implemented an Area-wide management (AWM) program consisting of male annihilation, protein baiting, sanitation and farmer training over 65 ha of mango in the districts of Indramayu (40 ha) and Cirebon (25ha). The harvested fruit goes then through a supply chain or series of different middlemen before it reaches the end point of sale to the consumer. A study was conducted from January 2020 to December 2021 in the major mango production districts of Cirebon and Indramayu, West Java, to determine the levels of fruit fly infestation from fruits sourced from the three different fruit fly management systems, and along the supply chain.

Methods: Two hundred mango fruits were collected randomly every month at harvest (October, November, and December) from each of the following areas: 1) The Area-Wide Management zone at Krasak, Indramayu (40 ha) and Sedong Lor, Cirebon (25 ha). 2) Farmer orchards that used methyl eugenol traps alone installed along the perimeter of mango orchards at Putat, Cirebon (45 ha). 3) Standard insecticide treated orchards at Pawidean, Indramayu (53 ha). 4) Traditional markets in Subdistrict Indramayu, Indramayu, and Subdistrict Dukuh Pantang, Cirebon and from fruit traders at Jatibarang, Indramayu and Weru, Cirebon. One hundred fruit were visually observed in situ and another 100 fruit were set up in rearing containers for three days and then dissected to observe fruit fly eggs and larvae.

Results: Mango fruits from the farms adopting AWM had the lowest levels of fruit fly infestation at 0.71-1.13%. In comparison, mango farmers that used only methyl eugenol traps were much more severely infested at 14.83-16.83%. In the standard insecticide treated orchards, fruit fly infestation was 17.67-23.67%. Fruits sampled from fruit traders was had 17.50% infestation, meanwhile fruits sampled from traditional markets had the highest level of infestation at 18.17-36.67%.

Conclusion: The AWM approach is highly effective and provides greater than 98% control of fruit flies in smallholder mango orchards. The widely used practice of methyl eugenol trapping of male flies alone was ineffective. High levels of infestation (36%) throughout the supply chain demonstrates that fruit fly control is very poor in West Java and infested fruits are reaching the market. Thus, to reduce these losses, AWM should be promoted and implemented by the mango industry in Indonesia.

Keywords: Fruit fly, mango, supply chains

Poster: Area-wide Integrated Pest Management and Action Programs

Infestation levels of *Anastrepha fraterculus, Ceratitis capitata* and *Drosophila suzukii* in different fruit species in the province of Entre Ríos, Argentina.

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Background: In Argentina, three species of fruit flies of economic importance are recorded: *Anastrepha fraterculus* and *Ceratitis capitata*, which belong to the Tephritidae family, and *Drosophila suzukii* from the Drosophilidae family. All three species are highly polyphagous, with a wide and overlapping range of host species, including commercial and native fruit species. The objective of the work was to evaluate the level of infestation and overlapping of the three fly species in three fruit species in the department of Concordia, Entre Ríos.
Methods: A. fraterculus, C. capitata and *D, suzukii* infestation patterns were evaluated from pupae obtained from blueberry (*Vaccinium corymbosum*), blackberry (*Rubus* sp.) and ubajay (*Hexachlamys edulis*). Blueberry has high economic importance in the region; blackberry it is a preferred host species for the three fruit fly species; and ubajayis a very common native species in the area. Fruit were collected in Northeast region of Argentina (Concordia) and were processed individually. Sampling was carried out during the fruiting season, every two weeks. The study involved: 1200 blueberries, 720 blackberries and 553 ubajay.
Results: We observed differences in the proportions of fruitsinfested by the 3 fruit flies in the different host species. In blueberry and blackberry we found a higher proportion of fruit infested by *D. suzukii* (6.5% and 64.3% respectively), while ubajay presented a higher level of infestation by *A. fraterculus* (with 59.7% of fruit infested followed by *C. capitata* with 5.8% of infested fruit). In addition, for the three species of host analyzed, we found fruit co-infested by two or three species of fruit flies.
Conclusions: This study showed that the three fruit species sampled here are affected by the three fruit flies species present in Argentina. Infestation patterns suggest potential differential preference among the three fruit fly species, although preference test shuoldbe crried out to determine this In addition, we found that the three fruit fly species studied here coexist in the area and share their hosts. This could generate scenarios of competition between them during the larval and adult stages.

Key Words: *Anastrepha fraterculus, Ceratitis capitata, Drosophila suzukii*, invasive species, coexistence, shared host, infestation levels, space- temporal.

Poster: Area-wide Integrated Pest Management and Action Programs

Kobo-Fly: A field data collection system for fruit fly surveillance

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In crop protection, the value of pest surveillance data depends critically upon its timeliness. In the case of pests, including fruit flies, the lag between trap servicing and digital capture of the data reduces the value of surveillance for management and real-time decision support systems for the farmers. It is especially the case if the window of opportunity for undertaking pest control is narrow. Yet, many teams still rely on manual trapping and paper and spreadsheet methods to record information in the field. To process data recorded manually in the field and make it ready for web-mapping, it must first be transcribed. Manual data transfer from paper to spreadsheet is prone to human errors. Data captured by numerous users or field technicians working as part of a larger project often lacks consistency in approach and often needs to be further manually reformatted. Data should then be parsed and cleaned to fix typographical errors and inconsistencies before being uploaded into a database. This time-consuming and labour-intensive process introduces significant time-delays and costs into the system, and is inherently error-prone throughout.

In the FF-IPM project, we have developed Kobo-Fly – an app based on the Kobotoolbox platform. Kobo-Fly is an attractive, simple-to-use system for collecting data in a robust, timely manner and storing it in a cloud-computing database. To ease adoption, we built the Kobo-Fly application to closely mimic traditional data collection workflows, but removing the pain points, enhancing user experience and saving time in manual data digitisation. Kobo-Fly can be easily tailored to system needs. The system proved equally suitable and relevant for field or laboratory capture of trap catch data.

For conventional trap data collection, Kobo-Fly uses two linked forms: one for trap deployment form and another for regular data entry. The Kobotoolbox system supports the capture of picture images and using barcoding. Kobo-Fly works with geospatial information and creates instant reports and maps. The data capture system can work offline, syncing when subsequently connected to the internet, and it is available for Android and iOS devices. The User Experience testing results for Kobo-Fly were highly favourable. In practice, the application reduced the data latency by two weeks, enabling manual trap data to be linked to an early-alert web-mapping system alongside e-trapping results. In this presentation we demonstrate the functionality and opportunities that the KoboFly system presents for data enumerators.

Keywords: data collection, Kobotoolbox, surveillance, app, smart farming

Poster: Area-wide Integrated Pest Management and Action Programs

Management of pest fruit flies using a spatio-temporal depiction of population fluctuation as a guide

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Background: This information has been commissioned under the Victorian Government's *Managing Fruit Fly – Regional Grants Program*.

Male Queensland fruit fly (Qff) trap location (address and GPS), type of plant in which the trap was placed and trap site land use (i.e. urban, peri-urban or rural) were tabulated against fortnightly trap captures for each site and local weather (temperature, rainfall, %rh and others). The objective was to discover patterns which could be used to describe past populations of Qff and predict their future spread, survival and build-up.

Methods: Standard Lynfield traps for Qff were deployed in regional Victoria. Traps were serviced weekly or fortnightly depending on the season. Numbers of captured insects were recorded and entered into a spreadsheet with rows denoting individual trap sites and columns showing total number of Qff trapped per assessment at each trap site. Each cell was coloured according to the number of Qff trapped. Yellow cells denoted 4 to 7 Qff/fortnight, orange: 8 to 15 Qff/fortnight, red: 16 to 23 Qff/fortnight) and purple: □ 24 Qff/fortnight).

Results: Patterns were detected in yearly/seasonal fluctuations in Qff proliferation and could be described based on location, weather and Qff/host phenology. Variations in expected patterns indicated adverse, beneficial or no-change impacts. These could be attributed to human-mediated control, Qff host plant removal programs, community awareness programs and weather.

Conclusion: A descriptive spatio-temporal array presenting fruit fly trapping data can depict Qff population fluctuations over time and across the landscape and to:

- Predict time and location of changes in Qff populations
- Indicate the presence/location of persistent Qff hot spots
- Prioritise timely and cost-effective Qff management strategies
- Assess Qff management projects including sterile insect technique
- Assess impacts of other stressors/facilitators on Qff survival

Keywords: Queensland fruit fly, Population, Monitoring, Prediction

Poster: Area-wide Integrated Pest Management and Action Programs

Management of the *Ceratitis capitata* (Wied.) in Western Australia over 125 years

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Background: The Mediterranean fruit fly (*Ceratitis capitata*, Wiedemann) was first found in Western Australia in 1896. Over the past 125 years the Western Australian government and the horticultural industry have made strenuous efforts to contain and eradicate Medfly.

Methods: We review the establishment, spread, control and eradication efforts made over several decades and the current management status.

Results:

- In 1896 and the following decades, monitoring was done by inspecting orchards for infested fruits. Since effective traps and lures were not available at the time, it is likely that dispersal of adult populations was well advanced before fruit infestations were detected. Sprays such as lead arsenate and sodium fluorosilicate mixed with molasses applied to kill adults were ineffective and by the 1930's Medfly had spread throughout the south west.

1. In the 1960's organophosphate insecticides (malathion, diazinon, fenthion and dimethoate) were applied as cover sprays to kill adults, eggs and larvae. Larvae and pupae in the soil were controlled by insecticide drenches.
2. From the 1970's protein hydrolysate mixed with insecticides improved kill since adults were attracted to feed on the protein and this reduced populations over large areas in eradication exercises. In the 1990's management by applying the mixture as 50 - 100 ml spots to the foliage minimised impacts on beneficial and non-target insects.
3. Research in the 2000's found spinosad and abamectin to be effective. Clothianidin and thiacloprid although effective were restricted because of impacts on non-target insects. Area wide management of Medfly is encouraged in all growing districts.
4. Eradication of Medfly in WA in the isolated Carnarvon area over a 10-year period from 1975 to 1985 included the use of the sterile insect technique when numbers were reduced by insecticide treatments below detectable levels. This work was not successful as quarantine controls in place to prevent reinfestation were breached. From 2015 to 2019, a pilot eradication program was trialled in Carnarvon but due to poor support by the community, was discontinued.
5. The only area that has been successfully maintained free from Medfly is the Ord River Irrigation Area in Kununurra. This is an isolated area with very few Medfly hosts and where small findings of Medfly can be immediately eradicated using protein baiting and fruit stripping.

Conclusion: Area freedom is successfully maintained in the Ord River Irrigation Area in Kununurra. In all other areas Medfly is managed by using a combination of control techniques including bait spray, cover spray, mass trapping and orchard hygiene.

Key words: History and spread, chemical cover sprays, bait sprays, eradication, area freedom.

Poster: Area-wide Integrated Pest Management and Action Programs

Management of the mango fruit fly, *Bactrocera dorsalis* by farmer-groups through surveillance, ovipositional deterrence and post harvest protocols

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Background: In India, *Bactrocera dorsalis* (Hendel) is the most important Tephritid on mango and causes loss up to 80%. Male annihilation using methyl eugenol traps combined with sanitation and bait splash was the earlier recommendation. This when widely adopted in mango belts of South India, resulted in >95% reduction in fruit fly infestation. With the ban of Dichlorovos and restricted availability of Malathion in India, two toxicants used on the plywood lures for trapping, efficacy and longevity of traps have diminished.
It has been further found under laboratory experiments that all males do not respond spontaneously to ME lures; in fact some males do not respond at all. This explained why in spite of intensive trapping, control was not 100%. This became a hindrance to export. Bait splash with 10% jaggery (a sugarcane by-product) with Malathion became unviable.

Methods: So a series of experiments were conducted at IIHR Bengaluru (13.1348° N, 77.4960° E) between 2014 and 2016 to find out a suitable ovipositional deterrent using botanicals (mainly neem) in the laboratory and field. These were validated in an area-wide manner in selected districts (Chikkaballapura (13.4355°N, 77.7315°E) and Kolar districts (13.1362°N, 78.1291°E) of Karnataka on cvs Alphonso and Totapuri, respectively in 2021 and 2022.)

Results: It was found that azadirachtin 3000ppm @ 3ml/litre and herbal liquid soap (Rashvee, IPRS) @ 3ml/litre, effectively prevented oviposition by gravid females to >90%. The traps @ 10/acre served as surveillance tool indicating the intensity of *Bactrocera dorsalis* and perhaps partially as a male annihilation tool. Thus the recommendation was deploying traps at mid-way maturity of fruits, followed by three pre-harvest botanical sprays, in an area-wide manner among contiguous farms. This effectively brought down infestation. Sampling at harvest showed that more than 95% fruits were free of fruit fly.
For exports, these fruits were further subjected to thermal water treatment (46°C for one hour, in thermostat tank for EU markets). For US markets, these were further irradiated at 400 Grays. These were adopted by farmer-groups formed for bigger markets, exports and for processing industries.

Conclusions: An integrated management combining surveillance para-pheromone traps, followed by three herbal (botanical) deterrence sprays gave >95% control. For exports, thermal disinfestation tanks (scaled up to two tonne capacity) followed by irradiation gave 100% control. Further these fruits were residue-free.

Keywords: Area-wide, *Bactrocera dorsalis,*India, Mango fruit fly, Tephritidae.

Poster: Area-wide Integrated Pest Management and Action Programs

Population Dynamics of Fruit Fly Species in Mango, Guava and Jujube Cultivation Lands of Myanmar

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Background: Mango, guava and jujube are the most important major fruit crops of Myanmar. They are native fruits that can grow well as natural vegetation throughout the entire country under numerous climatic conditions. *Bactrocera dorsalis* and *Bactrocera correcta* are serious destructive pests to mango, guava and jujube production in Myanmar. As surveillance of fruit fly population is an important part of pest control and the insect population dynamics change over time and environment, a proper surveillance system is needed to identify the type of fruit fly each year. The key objectives of this surveillance programme are to confirm the presence of the fruit fly population and determine the size of the infested area occupied.
Methods: Fruit Fly Surveillance Programme consists of seasonal monitoring for the presence of fruit fly through the use of methyl eugenol lure traps placed at high risk locations throughout the Mandalay Region. This programme includes the servicing of traps, collection, specie identification and providing accurate and timely reporting to our department. This surveillance programme was initiated in 2017 for detection and early warning of fruit fly incursions.
Results: In 2017, the population of *B. dorsalis* was higher than that of B. correcta in mango cultivation lands of Mandalay region. However, in guava and jujube cultivation lands, the population of *B. correcta* was higher than that of *B. dorsalis*. During 2017 to 2021, the population of *B. dorsalis* were gradually diminished. In 2021, *B. correcta* became the most abundant fruit fly specie in mango, guava and jujube cultivation regions of Myanmar. Moreover, population density of fruit flies were gradually increased over time.
Conclusion: Within a 5 years period, the population of *B. correcta* is steadily dominant over the population of *B. dorsalis*. In current, we are investigating the key factors influenced on the dominant population of *B. correcta*. Moreover, field surveillance and monitoring of fruit fly populations are carried out in mango, guava, jujube, banana, lime and typical almonds cultivation land of Mandalay region in Myanmar.

***Keywords*:** *B. correcta*, *B. dorsalis*, Male Annihilation Technique, Population Dynamics

Poster: Area-wide Integrated Pest Management and Action Programs

Project F³: Fruit Fly Free. Establishment and maintenance of fruit production areas free and under low prevalence of fruit fly (Diptera: Tephritidae) pests in Southern Africa

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Background: F³: Fruit Fly Free is a project funded by the Standards and Trade Development Facility (https://www.standardsfacility.org/) and is a collaboration between institutions from South Africa, Mozambique and Belgium. The project aims at developing a regionally harmonised framework for development, implementation and recognition of Pest Free Areas (PFAs) and Areas of Low Pest Prevalence (ALPPs) for regulated fruit fly pests of commercial fruit commodities in Mozambique and South Africa. The target fruit fly pests in the F³ project are *Zeugodacus cucurbitae*, *Bactrocera dorsalis* and *Ceratitis capitata*. *Zeugodacus cucurbitae* was found in northern Mozambique in 2013 and areas in other parts of the country were considered pest free. *Bactrocera dorsalis* is widespread in Mozambique but still has a restricted distribution in South Africa. *Ceratitis capitata* remains an important fruit fly pest of many fruit commodities particularly in South Africa.

Methods: Trapping surveys using Cuelure baited traps were carried out in Mozambique and in South Africa to demarcate PFAs for *Z. cucurbitae*. In South Africa, trapping surveys using Methyl Eugenol baited traps were carried out to demarcate PFAs in the southern and north western parts of the country. In both Mozambique and South Africa, specific fruit production areas were designated as ALPPs for *B. dorsalis* and are being monitored. In South Africa, additionally, fruit production areas in the southern parts of the country were designated as ALPPs for *C. capitata* and are being monitored. A protocol for preservation of fruit fly specimens detected and a protocol and decision tree for identification were developed.

Results: *Zeugodacus cucurbitae* was not detected in the southern part of Mozambique and in South Africa. In South Africa, the status of currently declared PFAs for *B. dorsalis* is still being maintained. In Mozambique, catches of *B. dorsalis* in designated ALPPs in Maputo, Inhambane and Manica never exceeded 1 fly per trap per day. In South Africa, low levels of *C. capitata* were maintained in areas under suppression of *C. capitata* through an area wide integrated pest management system, which includes the release of sterile male flies. An electronic multi-entry identification tool for 29 taxa of economic importance in Africa was converted into a mobile application and is downloadable for free.

Conclusion: PFAs for *Z. cucurbitae* in southern provinces of Mozambique and in South Africa continue to be monitored. Activities on ALPPs for *B. dorsalis* and *C. capitata* also continue in the two countries. The project will be concluded in 2023.

Keywords*:* fruit fly free areas, *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, southern Africa

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Samoan Fruit fly (Bactrocera spp., Diptera: Tephritidae) Area-Wide Approach Program.

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Background: Two economically important fruit flies (Diptera: Tephritidae) have been established on all islands of Samoa*: Bactrocera kirki* (Froggart) and *Bactrocera. xanthodes* (Broun). This twosome infests over 200 host fruits and represents one of the greatest bottlenecks to expansion and development of diversified agriculture in Samoa. The goal of the Fruit Fly Area Wide Pest Management project is to develop and integrate biologically based pest management approaches that will result in an area wide suppression and control of fruit flies throughout selected agricultural areas of Samoa. This program integrate more technologies into a comprehensive package that is economically viable, environmentally acceptable and sustainable.
Methods: Through cooperation with farmers, we implemented trainings through information presented and demonstrations on the use of one or more of the following management tools to reduce and control the population of the seven species of fruit flies that attack agricultural crops. These tools includes; ***(a)*** Host Fruit Surveillance, ***(b)*** Quarantine Traps, ***(c)*** Protein Pinnacle Bait Spraying, ***(d)*** Bagging, ***(e)*** Public Awareness Campaigns
Results: The expected results from the activities carried out over the period covered by this program estimate are:
Introduction of sustainable means of controlling fruit fly pest populations at the farm level.

- Fallen and rotten fruits were collected, recorded, burned or buried on ground to cease the development of fruit fly maggots that are already present in rotten and fallen fruits and for farmers to be able to harvest an un-infested clean fruit. A total of 5,793 fruit samples vary depending on the availability of fruits, and the number of pupae recovered was very low.
- A total of 562 pairs of Steiner traps were placed on farm sites in both Upolu and Savaii whereby *Bactrocera kirki* seems to be most common and in high numbers in all *cuelure* traps compared to other species with a total of 87,082 collected.
- The principle behind protein bait spraying is that all immature female fruit flies need to feed on protein in order to become sexually mature. Fruit flies that fed on this poisoned protein, die and as a result young female fruit flies are prevented from ovi-positing.
- Mature green fruits were bagged to observe and record the number of recovered fruits developed through to clean ripening stage. Five thousand five hundred and two (5,502) fruits were bagged at its mature green stage and 3,859 recovered through to ripening stage without infestation.

Farmer's Association strengthened with capacity and systems to manage an expanded HTFA facility and to promote market linkages for its members.

Increased awareness of farmers on domestic and export marketing opportunities for fruit and vegetables.

Conclusion: There is a need for this program to continue in order for Samoa to enjoy free infested fruits and for farmers to produce good quality fruits and fruity vegetables not just for local consumers but also for overseas markets.

Keywords: *Bactrocera kirki*, *Bactrocera xanthodes*, Fruit fly Area-Wide Approach, South Pacific

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The CUBE: strategies to control fruit fly in home gardens

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Background: Private properties with inadequate or no control of fruit flies are the "weakest link" in an area wide management approach. A study of community engagement to manage Mediterranean fruit fly (*Ceratitis capitata*, Medfly), in Western Australia found that training, education and advice are needed for people to participate in the collective control of Medfly. People's behaviour is motivated by internal needs and learned by reinforcements: knowledge provides individuals with the technical skills to deal with the pest and enable them to make informed decisions about control options. Adhering to a fruit fly management plan requires skills, time, and money. Here we developed an easy-to-follow strategy as part of a project exploring behavioural economics to increase landholder participation in the control of Medfly in the Peel Harvey region of Western Australia.

Methods: Following the review of various tactics to control/suppress fruit fly worldwide, we developed a set of strategies that households could use to manage Medfly on their property: *The CUBE Strategy*. Tactics covered fruit hygiene, control and fruit fly exclusion options. We also calculated the cost (money and labour) of each tactic. The CUBE was promoted through workshops, newsletters and webpages prepared by the Peel-Harvey Biosecurity Group.

Results: The CUBE sets out four straightforward tactics that households can follow to manage fruit fly: **C**lear fruit (garden hygiene), **U**se lure traps, **B**ait with organic insecticides, and **E**nhance protection with nets. Each tactic outlines the rationale (why), what to do (how), the cost, frequency and where to apply them. Most options included low and high cost alternatives to allow landholders a choice between a cheap but labour-intensive measure or a ready-made, but more expensive commercially available solution.

Conclusion: Detailed fruit fly control information provides people with cognitive resources to motivate them to work hard at something that they consider important. The CUBE strategy supplies home gardeners with clear, easy to follow options to reduce the damage caused by Medfly on their property. Explaining the when, why, what, how and the cost of each control measure is necessary for an effective area wide fruit fly management plan.

Keywords: area wide management, Medfly, strategy, community participation, knowledge

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The FlyCast: Nudging community participation to control Mediterranean fruit fly

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Background: Fruit orchards close to urban areas are at constant risk of invasion and reinfestation by Mediterranean fruit fly (*Ceratitis capitata*, Medfly) from surrounding urban and peri-urban areas. Attempts to control or eradicate plant pests by landholders often fail due to a lack of pest control by urban and peri-urban landholders. Medfly can only be effectively managed by concerted community action. This pilot project explores a novel approach to increasing landholder engagement to control Medfly in the Peel Harvey region of Western Australia. The use of psychological "nudges" can influence householders to change their behaviour in a way that benefits their neighbours.

Methods: Six towns in the Peel-Harvey region were assessed through an economic field experiment consisting of different Medfly information treatments. These involved website content, workshops, and the distribution of a newsletter designed to deliver social-norm messages or “nudges” specific to their towns. The message provided to landholders about fruit fly was that they were expected to control Medfly on their properties for their own and their neighbours’ benefit. A trapping grid was established over the towns and monitored by the Peel-Harvey Biosecurity Group between February 2020 to June 2022 to measure the effects of the behavioural interventions. Trapping data were presented to indicate how towns in the treatment region performed relative to each other in controlling fruit fly. In addition, landholders were given cognitive "nudges" and four straightforward Medfly control measures named *The CUBE Strategy*.

Results: Provisional results indicate that the information treatments reduced Medfly abundance in two out of the four treatment towns. The training and communication material aimed to motivate the control of Medfly through both self-interest and care for the community. The FlyCast newsletter identified communities that had relatively high numbers of Medfly, based on the trapping data, to “nudge” them to take more action.

Conclusion: The project established a behavioural economics intervention to control a damaging pest across a region. Motivating people through practical knowledge and ongoing updates are a good combination to trigger effective participation on fruit fly control. The lessons learnt about analysing trap data and community information are readily transferable to other pests.

Keywords: Medfly, haviour, participation, social nudges, knowledge

Poster: Social, Economic and Policy Issues of Action Programs

Global Burden of Crop Loss initiative - framework and partnerships

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Crop health is increasingly threatened by climate change and pests including insects, weeds and pathogens. Reducing crop loss is critical to addressing the rapidly growing demand for food, improving livelihoods, and strengthening value chains. In addition, improving yields reduces the need to clear land for cultivation, thereby lessening agriculture's potential negative impacts on forests, grasslands, and watersheds and mitigating contributions to greenhouse gas emissions.

FAO estimates that pests reduce global crop production by 20% to 40%. However, precise data on the magnitude and causes of yield loss tend to be outdated, lack broad spatio-temporal coverage and are often based strictly on expert opinion. While crop loss due to biotic and abiotic factors causes significant impacts on food systems globally, we lack robust, actionable evidence on the problem. If we were able to know how much of our food supply we are losing, where we are losing it, and what we are losing it to, then actors across the plant health system could be empowered to take informed action to tackle the most critical problems in their regions.

The Global Burden of Crop Loss brings together people, data and ideas to work collaboratively on developing a data-driven methodology to help to quantify the magnitude of yield loss by crop, geography and attribute it to specific biotic or abiotic causes. It aims to provide rigorous, authoritative evidence on the impacts, causes, and risk factors of crop loss. We outline our approaches to quantify yield loss in major crops as a difference in attainable yield in context (attainable yield given local abiotic and socio-economic limitations), and reported crop production data, as well as data and approaches for apportioning losses to specific factors.

Inspired by the Global Burden of Disease in human health, a system that provides comprehensive data on the impact of hundreds of diseases and has transformed the public health agenda, we aim to have a similarly transformative impact on plant health. Evidence-based decision making across the plant health system will enable resources and efforts to be directed where they are most needed, resulting in more targeted research and better investments, and will enable evaluating the effectiveness of interventions.

Keywords: crop loss, yield, crop health, pest impact, burden

Poster: Social, Economic and Policy Issues of Action Programs

Participatory approach for modeling fruit fly injuries on chayote in Réunion

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Background: Chayote is a crop traditionally cultivated in Réunion, but it is subject to numerous attacks by fruit flies, in particular *Dacus ciliatus* (Loew). During the last decade, experiments of agroecological management of fruit flies, in which farmers have been the central actors, have allowed to strongly limit the use of insecticides on chayote. In order to better support farmers in the management of fruit flies in their fields, a participatory approach aimed at designing and developing a model for predicting fruit fly injuries on chayote crops.
Methods: From 2015 to 2018, observations were made on fruit fly injuries in different plots in Salazie, the main chayote production area in Réunion. These observations focused on different criteria: geographical location and altitude of the plots, cultivation practices, phytosanitary practices in general and on fruit flies in particular, farmers' practices in the vicinity of the plots, fly injuries. A qualitative model, based on the IPSIM approach, was designed and built in a collective manner, with farmers playing a central role in this process. The construction of the model, its evaluation, as well as the construction of a user-friendly online interface were carried out by research organizations (Cirad, INRAE). The assessment of this model was carried out with a large panel of farmers.
Results: From the design of the model, farmers, agricultural advisors, trainers, public policy makers and researchers collaborated to define the conceptual scheme behind the IPSIM-chayote model that was developed. The fact that farmers were involved from the design phase of the model and that they were actors in the assessment phase allowed for their rapid appropriation and autonomous use of the tool. Farmers can now predict fruit fly injuries on their plots themselves; they can also improve their knowledge of the biology and ecology of fruit flies and of Agroecological Crop Protection practices. Extension organizations, such as the Chambre of Agriculture, use this model during the training sessions they provide to farmers. IPSIM-chayote is available online https://pvbmt-apps.cirad.fr/apps/ipsim-chayote/?lang=en in three languages.
Conclusions: The originality of this qualitative IPSIM-chayote model is that it was designed, built and evaluated using a participatory approach in which farmers played a central role. They therefore easily appropriated this model to manage their plots and improve their knowledge. This model can be seen as an innovative example to collectively build models for other crops or other pests in order to promote the implementation of Agroecological Crop Protection.

Key Words: Agroecological Crop Protection, modeling, participatory approach