



12th International Symposium on Fruit Flies of Economic Importance

PRELIMINARY
ABSTRACT BOOK

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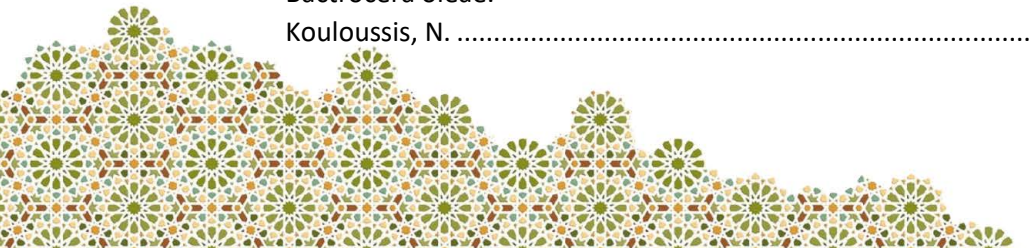
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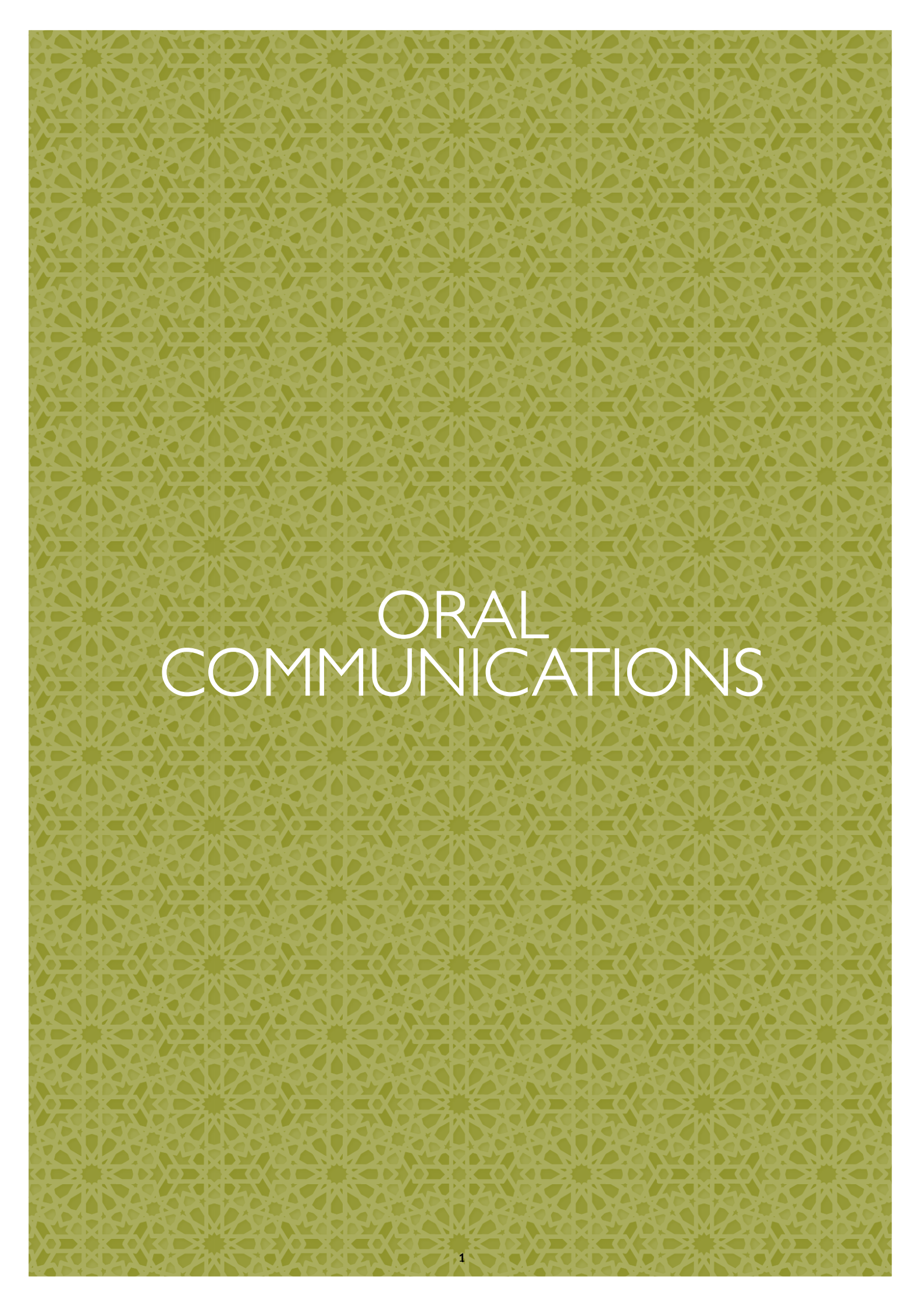
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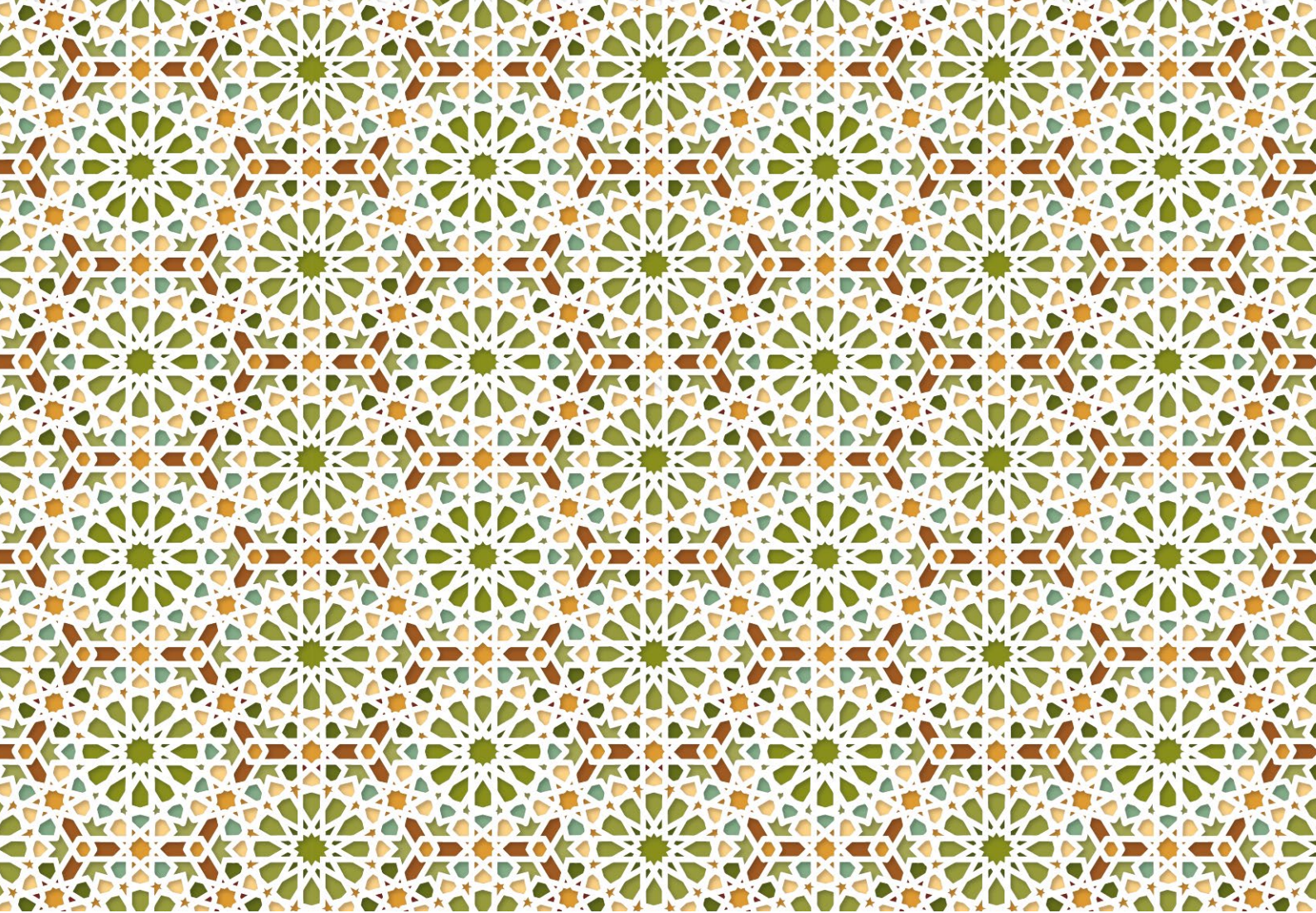
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Biology, Ecology, Physiology and Behavior

Evidence for a reproductive arrest in *Bactrocera tryoni*

Anthony R. Clarke¹ and Peter Leach²

1. School of Biology & Environmental Science, Queensland University of Technology, GPO Box 2434, Brisbane City, QLD 4001, Australia. E-mail: a.clarke@qut.edu.au

2. Horticulture and Forestry Science, Department of Agriculture and Fisheries, P.O. Box 652, Cairns, QLD 4870, Australia. Email: peter.leach@dip.qld.gov.au

Background: It is assumed that polyphagous *Bactrocera* will breed continuously if hosts and temperature are not limiting. Despite this, the Queensland fruit fly (*Bactrocera tryoni* (Froggatt)) shows repeatable declines in its seasonal abundance, with the fly rare in traps from May to August in both tropical and temperate areas. While these are the Australian winter months when such declines might be expected in temperate areas, winter in the tropics is a relative term and mean temperatures remain above the species' developmental thresholds. To better understand this seasonal decline, we carried out field, laboratory and literature research on *B. tryoni*'s phenology and reproductive biology.

Methods and Results: At a subtropical site where hosts were always present and temperature-driven models predicted seven generations per year, we used the captive-cohort method to age wild fly populations. We established that only two, rarely three, generations were completed in a year with breeding absent from May to September. Unexpectedly, longevity of the reference cohorts in the laboratory varied with time of year. Using mixed-sex cohorts of flies supplied with sugar and protein and held outdoors, we subsequently demonstrated that females emerging in May did not develop eggs until mid-July, nor hold sperm in spermathecae until mid-August. Even with the milder temperatures, day-degree accumulation should have allowed sexual maturity within four weeks, not the 12 weeks recorded before mating was seen. Further, females which survived from autumn through to spring and were by then four months old, laid >70% of the egg load of 14 day old new-generation flies, and those eggs were equally viable.

Conclusions: Many tropical Diptera, including *Drosophila*, exhibit periods of reproductive dormancy to survive the monsoonal dry season when breeding hosts are rare. A review of literature found *B. tryoni* 'ticked all the boxes' for a species exhibiting such dormancy during the May-August period, including non-vitellogenic immature ovaries, increased stress resistance, reduced metabolism and increased lifespan. Thus, data strongly suggests that *B. tryoni* has a facultative reproductive diapause. We argue this reproductive physiology is adaptive for surviving the dry season of the monsoonal tropics but still expresses itself in human-modified habitats. *Bactrocera dorsalis* shows a similar seasonal phenology to *B. tryoni*, although reversed in the northern hemisphere, and may thus have a similar reproductive physiology.

Key words: *Bactrocera tryoni*, population phenology, diapause, reproduction

Ecological niche modeling of the melon fly (*Zeugodacus cucurbitae*) to assess its potential establishment in Southeast Africa

Luis Bota, Diana Quintero, Dercio Romão, Elemia Chidakwa, Laura Canhanga, Lore Esselens, Marc De Meyer, Domingos Cugala, Massimiliano Virgilio

National Fruit Fly Laboratory of Mozambique, Pigivite Road 678, PoBox 42, Chimoio, Mozambique, Email: luisbota@yahoo.com.br

Background: Melon fly (*Zeugodacus cucurbitae*) is a highly invasive tephritid pest of cucurbit crops and represents a growing threat to horticultural production in Southeast Africa. Despite its continued spread in the region, large areas remain pest-free, highlighting the need for spatially risk assessments to support early detection and preventive management.

Methods: We assessed the potential climatic suitability of *Z. cucurbitae* in Southeast Africa using Ecological Niche Models (ENMs) calibrated with global occurrence records and informed by experimentally derived physiological temperature limits. Maximum Entropy (MaxEnt) and Boosted Regression Trees (BRT) models were applied using WorldClim bioclimatic variables. Multicollinearity was addressed through variance inflation factor (VIF) screening, and model performance was evaluated using cross-validation. Model outputs were projected to Southeast Africa under a physiological temperature mask to restrict predictions to biologically plausible environments.

Results: Both models showed high discriminatory performance, indicating robust predictive ability. The optimized MaxEnt model achieved a mean cross-validated AUC of 0.878 ± 0.005 , while the BRT model reached a cross-validated AUC of 0.938 ± 0.0066 . Correlations between predicted and observed values were 0.810 ± 0.010 for MaxEnt and 0.804 ± 0.0173 for BRT. Predicted suitability maps consistently highlighted eastern and northern Mozambique, southern Tanzania, and parts of Malawi as areas of high to optimal climatic suitability. In contrast, southern Mozambique, South Africa, eSwatini, Lesotho, Zimbabwe, and Botswana were largely classified as low suitability or excluded by physiological constraints. Across both modelling approaches, mean diurnal temperature range (Bio2), isothermality (Bio3), and precipitation seasonality (Bio15) were the most influential environmental predictors.

Conclusions: The convergence of results across modelling algorithms shows the reliability of the predicted suitability patterns and highlights key climatic drivers shaping the potential distribution of *Z. cucurbitae* in Southeast Africa. These findings provide a scientific base to support surveillance prioritization, early warning systems, and regional pest management strategies, particularly in areas currently free of the pest but climatically suitable for establishment.

Key words: Melon fly, *Zeugodacus cucurbitae*, ecological niche modelling, MaxEnt, Boosted Regression Trees.

Geographic variation and acclimation capacity shape thermal tolerances of the Mexican fruit fly, *Anastrepha ludens*

Liqiong Zhang, Vasilis G. Rodovitis, Daniel A. Hahn, Jhonatan S. Aguilar, Christian Stauffer, Teresa M. Vera, Rui Pereira, Scott W. Myers, Corinna S. Bazelet, & Vanessa S. Dias

Insect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Wagramer Strasse 5, 1400 Vienna, Austria. E-mail: Liq.Zhang@iaea.org

Background: Because climate change may facilitate the geographic range expansion of many insect pests, understanding their thermal biology has become critical for predicting invasion potential and improving pest management strategies. Geographic variation in thermal tolerance has been documented across multiple insect species and is frequently associated with genetic differentiation among populations. In addition, thermal tolerance is a phenotypically plastic trait that can be affected by the insects' thermal history, particularly through acclimation. The Mexican fruit fly, *Anastrepha ludens*, is a key pest species distributed across different eco-climatic regions in Central America and Mexico, for which population-level thermal biology remains insufficiently characterized. In particular, thermal tolerance and acclimation capacity have not been studied in *A. ludens* populations coming from different geographical origins and climatic conditions. The aim of this study was to evaluate thermal tolerances among multiple *A. ludens* populations and to assess how acclimation modulates these responses.

Methods: We compared the thermal limits of 15-day-old adults from three climatically different *A. ludens* populations from Belize, Guatemala, and Mexico (Tamaulipas) that were acclimated at 15 °C or 25 °C for 4 (from 11-days-old to 15-days-old) or 7 days (from 8-days-old to 15-days-old). Thermal tolerance was assessed by measuring Critical Thermal Minimum (CT_{min}), Critical Thermal Maximum (CT_{max}), and Chill Coma Recovery Time (CCRT).

Results: Cold acclimation at 15 °C for 7 days significantly reduced the CT_{min}, CT_{max}, and CCRT values of all *A. ludens* populations, indicating improved cold performance and reduced heat performance compared to adults acclimated at 25 °C. In addition, thermal tolerances differed significantly among *A. ludens* populations, with the Mexico population exhibiting the lowest CT_{min} and the Belize population the highest CT_{max}. Moreover, *A. ludens* adults from a subtropical region of Mexico exhibited greater thermal plasticity in response to cold acclimation than *A. ludens* from a tropical region of Belize.

Conclusions: Our results demonstrated the presence of geographic variation in thermal tolerance and acclimation capacity of *A. ludens*. Further research should focus on how such variation and plasticity can impact the invasive potential, pest distribution, and management of this pest.

Key words: climate change, thermal biology, thermal plasticity, invasive potential

Effects of larval nutrition on chill-coma recovery in *Anastrepha fraterculus* adults

Fátima L. Fernández^{1,2,3}, M. Josefina Ruiz², Pilar Medina-Pereyra⁴, M. Rosario Jorge³, Diego F. Segura^{5,6}, Alejandra C. Scannapieco^{2,5}, David Nestel⁷, M. Teresa Vera^{1,2,8} & Lucía Goane^{1,2}

¹Facultad de Agronomía, Zootecnia y Veterinaria, UNT, Tucumán, Argentina; ²CONICET, Argentina; ³Facultad de Ciencias Naturales e IML, UNT, Tucumán, Argentina; ⁴Instituto de Fisiología Animal, Fundación Miguel Lillo, Tucumán, Argentina; ⁵Instituto de Genética (INTA), GV-IABIMO (CONICET), Hurlingham, Argentina; ⁶Instituto de Investigación en Ciencias Agrarias y Veterinarias, Universidad del Salvador, Argentina; ⁷Institute of Plant Protection, Entomology Dept., ARO Volcani Ctr., Beit Dagan 50250, Israel; ⁸Insect Pest Control Section, Joint FAO/IAEA Centre, Dept. of Nuclear Sciences and Applications, Vienna (current address), Austria. E-mail: flfernandez@csnat.unt.edu.ar

Background: Chill-coma recovery time is an indicator of cold tolerance with important implications for the success of the sterile insect technique (SIT): particularly when chilled-adult releases are implemented. Although larval nutrition is known to influence several life-history traits, but its role in adult cold tolerance remains poorly understood. Here, we investigated how larval diet affects adult cold tolerance by measuring chill-coma recovery time as an indicator of thermal tolerance in the South American fruit fly, *Anastrepha fraterculus*, a key pest in many South American countries.

Methods: Three artificial larval diets were formulated using a common base of non-hydrolysed brewer's yeast and sugar, supplemented with: (1) wheat germ and agar; (2) corn flour; and (3) wheat germ and mashed carrot. Chill-coma recovery was assessed in two independent experiments by exposing newly emerged and 7-day-old adults to 0 °C for 8 h. Post-recovery longevity was assessed in newly emerged males and females, whereas pheromone calling behaviour was evaluated in recovered 7-day-old males over a three-day period. Chill-coma recovery time was analysed using parametric Weibull models and Kaplan–Meier survival curves. Post-recovery longevity and calling behaviour were analysed using generalized linear mixed models (GLMMs).

Results: Larval diet, sex, and their interaction significantly affected chill-coma recovery time. Newly emerged males and 7-d-old females reared on the carrot-based diet showed the shortest recovery times. Larval diet did not significantly affect post-recovery longevity. Larval diet and age significantly influenced the calling behaviour of male. Males grown as immatures in the carrot diet reached maximal calling rates one day earlier (8 days of post-emergence) than those reared on wheat germ (9 days of post-emergence). Calling behaviour also differed between chill-coma recovered individuals and controls, with recovered flies generally exhibiting higher calling rates.

Conclusions: Our findings underscore the relevance of larval nutritional composition as a tool to improve adult performance after cold stress. The observed variation in chill-coma recovery suggests that optimizing larval diet could improve adult tolerance to the low-temperature exposure experienced as part of the release process, potentially enhancing the efficiency of SIT programs. Future research should focus on whether these nutritional benefits persist following ionizing radiation, as the physiological stress of irradiation may interact with larval nutritional history.

Key words: larval diet, South American fruit fly, cold tolerance, SIT programs

Interspecific larval competition and invasiveness: conditions mediating the interactions between *Bactrocera zonata* and *Ceratitis capitata* in fruit

Hamburg, Mai^{1,2}, Yuval, Boaz¹, Delatte, Hélène³, Hafzi, Abir^{3,4}, **Ben Yosef, Michael²**

¹Hebrew University of Jerusalem, Israel; ²Agricultural Research Organization, Israel | michaelb@volcani.agri.gov.il; ³Centre de coopération internationale en recherche agronomique pour le développement, La Réunion, France; ⁴Université de La Réunion, La Réunion, France.

Background: Polyphagous fruit flies of the genus *Bactrocera* (Tephritidae) have been involved in several recent invasions, leading to changes in endemic fruit fly fauna and negative economic consequences. One such species, the peach fruit fly (*B. zonata*, *Bz*) is currently at risk of establishing in some areas of Mediterranean Europe, where its potential biogeographic range overlaps with that of the Mediterranean fruit fly (*Ceratitis capitata*, *Cc*). Considering the attenuating effect of interspecific competition on population expansion, we examined the interaction between the two species during larval development, hypothesizing that competitive outcomes are temperature-dependent, favor older larvae over younger heterospecific competitors, and vary with diet composition.

Methods: We tested the effects of temperature (20°C and 30°C), diet (mango, and mango supplemented with yeast as a simulation of nutrition provided by microbes) and age difference between larvae (same age and ± 1 day difference between heterospecifics) on the competition between the two species. All combinations of these variables were addressed and effects on egg-adult survival rate, pupal weight, and development time were quantified. Rearing on fruit-based media allowed control over the available resources (g diet/larva).

Results: *Bz* survival remained high and was unaffected by *Cc* larvae. Contrarily, *Cc* survival was lower and significantly reduced by older *Bz* larvae. Both flies survived better on a diet containing microbes at 20°C, but not at 30°C. Regardless of species, development time and pupal weight were not affected by competition but varied according to diet and temperature. Overall, *Bz* remained competitively superior to *Cc* (measured by their relative proportions in the emerging adults); however, *Cc* competed better on a diet containing microbes and matched *Bz* only at 20°C when given an age advantage.

Conclusions: Our experiments point to the competitive advantage of *Bz* over *Cc* and suggest that physiological, temperature-related differences in the capacity to extract protein from the diet affect the relative competitiveness of the two species. These results may be considered with existing ecological data to estimate the future spatial and temporal distribution of the two flies in the Mediterranean basin.

Key words: invasiveness, *Bactrocera*, *Ceratitis*, interspecific competition, larvae

Divergent cold tolerance and plasticity in Tephritid fruit flies: A comparative approach

Vasilis G. Rodovitis^{1,2}, Jhonatan S. Aguilar², Georgia D. Papadogiorgou¹, Cleopatra A. Moraiti¹, Paraschos N. Prekas¹, Liqiong Zhang², Teresa M. Vera², Polychronis Rempoulakis², Rui Pereira², John S. Terblanche³ and Nikos T. Papadopoulos^{1*}

¹ Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Volos, Greece

² Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, Vienna, Austria

³ Department of Conservation Ecology and Entomology, Faculty of AgriSciences, Stellenbosch University, Stellenbosch, South Africa

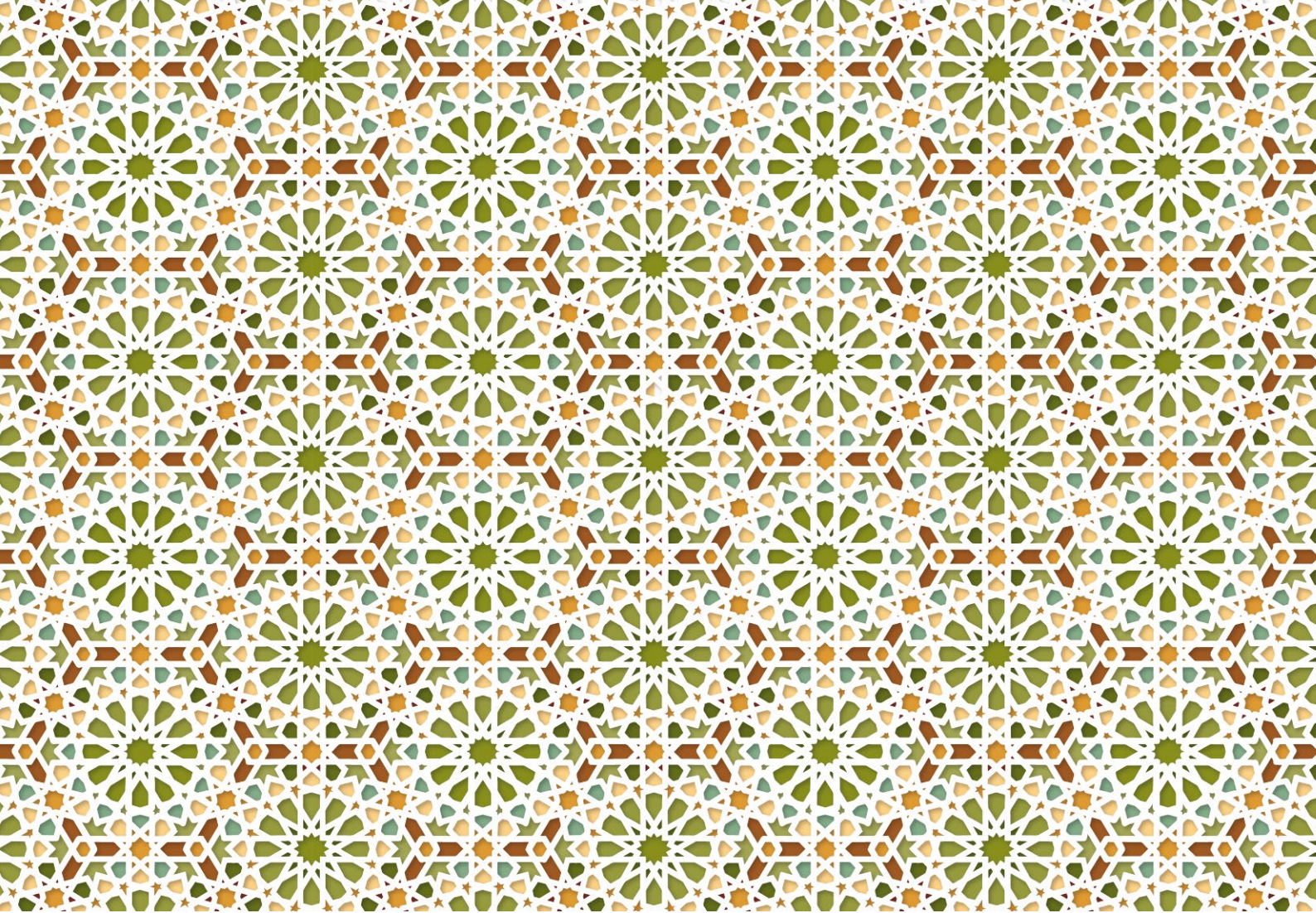
Background: Cold tolerance often represents a critical barrier to establishment and persistence of invasive insects of tropical origin in temperate regions. Phenotypic plasticity in thermal traits, particularly in response to prior thermal experience, may enhance invasion success, yet comparative studies across different related taxa remain limited. In true fruit flies (Diptera: Tephritidae), which include some of the world's most economically important invasive pests, interspecific variation in cold tolerance and the extent to which plastic responses differ among genera are still poorly understood. We evaluated cold tolerance and thermal plasticity in 12 species from four genera: six *Bactrocera* (*B. correcta*, *B. cucurbitae*, *B. dorsalis*, *B. oleae*, *B. tryoni*, *B. zonata*), four *Anastrepha* (*A. fraterculus*, *A. ludens*, *A. obliqua*, *A. suspensa*), *Zeugodacus tau*, and *Ceratitis capitata*, originating from diverse biogeographic regions worldwide.

Methods: Adults of all above species were acclimated for five days at 15, 20, 25, or 30 °C and Critical Thermal Minimum (CTmin) and Chill Coma Recovery Time (CCRT) was recorded at day 11.

Results: All species exhibited pronounced plastic responses to acclimation temperature, with colder acclimation generally resulting in lower CTmin values and faster Chill Coma Recovery. *Bactocera tryoni* showed the lowest CTmin overall, followed by *B. zonata* and *A. fraterculus*. Within *Bactocera* species, CTmin increased from *B. tryoni* to *B. zonata*, then *B. correcta*, *B. cucurbitae*, *B. oleae* and *B. dorsalis*, while *A. fraterculus* was the most cold-tolerant species within the *Anastrepha* species tested. In contrast, CCRT exhibited lower plasticity. Although effects of acclimation were evident across all taxa, *Anastrepha* species generally showed greater CCRT plasticity compared to other genera. Within *Bactocera* species, *B. dorsalis* and *B. cucurbitae* expressed greater levels of plasticity. *Zeugodacus tau* recovered fastest from chill coma, followed by *B. cucurbitae* and *B. correcta*.

Conclusions: These findings reveal distinct cold tolerance strategies among tephritid fruit flies and underscore acclimation temperature as a key determinant of adult cold performance and plasticity. Incorporating thermal plasticity into bioclimatic and pest risk assessment models will improve predictions of invasion success, establishment probability, and range expansion, particularly by better capturing overwintering dynamics under climate change.

Key words: acclimation; phenotypic plasticity; chill coma recovery; critical thermal minimum; invasion biology



ORAL COMMUNICATIONS

Taxonomy and Morphology

BIODIVERSITY OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN MOROCCO AND THEIR HOST PLANT ASSOCIATIONS

Younes EL HARYM*, Boutaïna BELQAT

ILESCB URL-CNRST N°18, FS, Abdelmalek Essaadi University, Tetouan, Morocco

✉ elharym.younes@gmail.com; <https://orcid.org/0000-0002-6852-6602>

✉ belqat@gmail.com; <https://orcid.org/0000-0003-2857-7699>

**Corresponding author*

Background: Fruit flies (Diptera: Tephritidae) are one of the largest families of acalyptrate flies, with 5000 species worldwide. Many species, particularly within the subfamily Tephritinae, develop on plants of the family Asteraceae. Numerous species infest flower heads, seeds, stems, or roots of Asteraceae causing significant damage, while others develop on flowers and leaves of additional plant families such as Lamiaceae, or directly attack fruits. Tephritidae are of agro-economic importance and include some of the most widespread agricultural pests in Mediterranean countries. Infestations by fruit flies result in deterioration of fruit quality, notably through increased acidity, leading to substantial yield losses that may exceed 80% of the harvest, stressing the importance to know these pests. However, the family Tephritidae does not include only harmful species; it also comprises species that are harmless or even beneficial.

Method: A first comprehensive study is currently being conducted in Morocco with the aim of assessing the biodiversity of Tephritidae, analysing host plant-species interactions, and distinguishing economically harmful species from those that are harmless or beneficial. Our study was based on more than 200 survey sites distributed across several mountainous regions of Morocco, including the Rif, Coastal Meseta, Beni Snassen, Middle Atlas, High Atlas, and Anti-Atlas, surveyed between 2013 and 2025.

Results: These surveys resulted in the collection of more than 3979 adult Tephritidae individuals (2061 males and 1918 females). The collected specimens were classified into three subfamilies, ten tribes, and thirty genera. Taxonomic analysis of these genera led to the identification of 61 species. In addition, the checklist of Moroccan Tephritidae was supplemented with 13 species previously reported in the literature.

Conclusion: One of the main objectives of this study was the development of a comprehensive catalogue compiling all Tephritidae species known from Morocco, together with their associated host plant ranges.

Keywords: Biodiversity, catalogue, Diptera, pest species, new species, fruit fly, host plant, Morocco.

Comparative transcriptomic analyses provide insights into the ecological driving force behind odorant receptor evolution in Tephritidae

Emma Persyn^{1,2}, Pierre-François Duyck^{3,4}, Marie-Christine François², Christian Mille³, Vincent Jacob¹, **Emmanuelle Jacquin-Joly**²

¹CIRAD, Université de la Réunion, UMR PVBMT, 7 ch. de de l'IRAT, F-97410 Saint-Pierre, La Réunion, France

²INRAE, Sorbonne Université, CNRS, IRD, UPEC, Université Paris Cité, Institute of Ecology & Environmental Sciences of Paris, F-78026 Versailles cedex, France

³IAC, Institut Agronomique néo-Calédonien, Equipe ARBOREAL, Laboratoire d'Entomologie Appliquée, Station de Recherches Fruitières de Pocquereux, F-98880 La Foa, New Caledonia

⁴CIRAD, UMR PVBMT, F-98488 Nouméa, New Caledonia

E-mail: emmanuelle.joly@inrae.fr

Background: The insect olfactory system has evolved to guide species toward specific mating partners, food sources, and oviposition sites. However, how species-specific repertoires of odorant receptors (ORs)—which mediate the detection of volatile cues—have been shaped by ecological pressures remains poorly understood. Fruit flies of the Tephritidae family (Diptera) exhibit highly diverse host preferences due to multiple host switches throughout their evolutionary history, making them ideal models to explore this question.

Methods: In this study, we used RNA sequencing to manually identify the OR repertoires of thirteen Tephritidae species with varying host ranges: *Bactrocera curvipennis*, *Bactrocera dorsalis*, *Bactrocera psidii*, *Bactrocera tryoni*, *Bactrocera umbrosa*, *Bactrocera zonata*, *Ceratitis capitata*, *Ceratitis catoirii*, *Ceratitis quilicii*, *Dacus ciliatus*, *Dacus demmerezii*, *Neoceratitis cyanescens*, and *Zeugodacus cucurbitae*, and conducted differential expression analyses. We next searched for significant correlations among OR characteristics, species host range, and phylogenetic position.

Results: We annotated 60-80 OR transcripts per species, including the obligatory coreceptor *Orco*, and identified a total of 698 new ORs. Differential expression analyses revealed some organ- and sex-biased OR expression patterns. Importantly, after correcting for phylogenetic distance, we found significant correlations between certain OR repertoire characteristics and host range. Specifically, both the sequence and relative expression levels of several ORs were more conserved in polyphagous than in oligophagous species, and certain ORs were uniquely present in polyphagous species.

Conclusions: This work brings new resources for the fruit fly community and provide molecular insights into the ecological forces shaping OR evolution in Tephritidae fruit flies.

Key-words: Odorant receptors, RNAseq, Phylogeny, Differential expression, Host range

Thermal plasticity is regulated by a key microRNA during range expansion of *Bactrocera dorsalis* in China

Zhihong Li¹, Yan Zhao^{1,2}, Juntao Hu³, Juan Du¹, Shaokun Guo¹, Youming Hou²

¹State Key Laboratory of Agricultural and Forestry Biosecurity, MARA Key Lab of Surveillance and Management for Plant Quarantine Pests, College of Plant Protection, China Agricultural University, Beijing 100193, China. E-mail: lizh@cau.edu.cn

²State Key Laboratory of Agricultural and Forestry Biosecurity, Fujian Agriculture and Forestry University, Fuzhou 350002, China

³Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, Center for Evolutionary Biology, School of Life Sciences, Fudan University, Shanghai 200433, China

Background: Invasive species often exhibit rapid adaptation to novel thermal environments during range expansion, but the underlying molecular mechanisms remain poorly understood. Phenotypic plasticity is considered a key driver of such adaptation, yet the role of post-transcriptional regulation, particularly by microRNAs (miRNAs), in shaping thermal plasticity during invasion is largely unexplored. We investigated the thermal adaptation mechanisms of the invasive Oriental fruit fly, *Bactrocera dorsalis*, which has expanded northward in China, encountering colder and more variable climates.

Methods: We compared southern (ancestral) and northern (invasive front) populations under cold (17°C) and warm (32°C) acclimation. Phenotypic traits (critical thermal limits, body size, and mass) were measured, and integrated transcriptomic and miRNA-seq analyses were performed on head and muscle tissues. Weighted Gene Co-expression Network Analysis (WGCNA) was used to identify modules associated with thermal tolerance. Functional validation of key miRNA–mRNA interactions was conducted using dual-luciferase reporter assays and RNAi-mediated knockdown.

Results: Northern populations showed reduced phenotypic plasticity but higher basal cold tolerance compared to southern populations. Transcriptomic analysis revealed genetic assimilation of “frontloaded” genes in the north, where genes constitutively expressed in northern populations were only induced under acclimation in southern populations. A key cold-tolerance module was identified, with the chitin-binding gene *thw* as a central hub. miRNA sequencing revealed miR-276b as a thermally responsive regulator, directly targeting *thw*. Functional experiments confirmed that miR-276b knockdown increased cold tolerance, while *thw* knockdown impaired it, demonstrating a conserved miRNA-mediated regulatory axis.

Conclusions: Our study demonstrates that reduced plasticity at the invasion front is associated with genetic assimilation of thermally responsive genes. We identify a novel regulatory pathway in which miR-276b modulates *thw* expression to enhance cold tolerance in *B. dorsalis*. These findings highlight the importance of

miRNA-mediated post-transcriptional regulation in facilitating rapid thermal adaptation and range expansion during biological invasions, providing insights into the molecular basis of climate-driven invasion success.

Key words: thermal plasticity, genetic assimilation, microRNA, range expansion, invasive species, *Bactrocera dorsalis*

***Bactrocera oleae* gut microbiome as a resource to improve insect mass rearing**

Aya Elsayed¹, Silvia Romagnolo¹, Kostas Bourtzis², Alberto Alma¹, Elena Gonella¹

¹ Department of Agricultural, Forest and Food Sciences, University of Torino, Italy

² Insect Pest Control Subprogramme, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, P.O. Box 100, 1400 Vienna, Austria

E-mail: aya.elsayed@unito.it

Background: Insect diversity and evolutionary success are significantly influenced by their interactions with beneficial microorganisms. The gut microbiome is essential for nutrition, digestion, immunity, and overall health in insects. The olive fruit fly, *Bactrocera oleae* (Rossi), a major pest in olive cultivation, has a gut microbiome dominated by the symbiont '*Candidatus* *Erwinia dacicola*'. Laboratory rearing conditions lead to symbiont loss, reducing insect fitness critical for sterile insect technique (SIT) programs.

Methods: We conducted transcriptomic sequencing of the gut microbiome in a field-collected population and its lab-reared F1 generation to identify differentially expressed genes (DEGs) in response to the shift from natural to artificial diet; additionally, taxonomy assignment was conducted to determine microbial composition of the insect guts, and relevant bacteria were isolated in pure cultures. Since a slight reduction of *E. dacicola* was recorded in the lab-reared generation, the long-term symbiont loss was simulated through antimicrobial treatment, and the performance of treated flies was compared to that of control flies. Moreover, qPCR was employed to compare the abundance of active *E. dacicola* with and without anti-symbiont treatment.

Results: Our results confirmed that *E. dacicola* is a core member of the olive fruit fly gut microbiome, as its reduction resulted in DEGs linked to nutrient metabolism, stress response, and immune function. Antimicrobial treatment significantly reduced the abundance of *E. dacicola* and the fly performance. Moreover, the *E. dacicola* decrease in the gut microbial composition of the F1 generation corresponded to a replacement by other taxa, suggesting a compensatory role. A total of 103 common metabolic pathways were identified between the main symbiont and the bacterial isolates. *Stenotrophomonas* isolates exhibited augmented coenzyme A production pathways, whereas *Enterobacter* isolates had superior carbohydrate processing capabilities. To test this hypothesis, we assessed the impact of redelivering isolated gut bacteria, which were abundant in the F1 microbiome, on long-term lab-reared *B. oleae*, by incorporating these microbes into the fly diet. Several microbial isolates were shown to increase the fly performance.

Conclusions: These findings underscore the dynamic nature of the olive fruit fly gut microbiome and the critical role of *E. dacicola* in maintaining its balance. Additionally, functionally equivalent culturable bacteria may serve as substitutes for the uncultivable obligate symbiont *Ca. E. dacicola* to improve insect performance.

Key words: *Bactrocera oleae*, Gut microbiome, Insect's fitness, *E. dacicola*

ATTRACTION OF *Anastrepha ludens* TO VOLATILE COMPOUNDS EMITTED BY FIVE MANGO CULTIVARS

Judith Velazquez^{1,2}, Dina Orozco^{1,2}, Julio Rojas², Leopoldo Cruz-López², **Pablo Liedo**²

¹*Instituto de Biociencias, Universidad Autónoma de Chiapas. Boulevard Príncipe Akishino s/n, Col. Solidaridad 2000, Tapachula 30798, Chiapas, México.*

²*Departamento de Ecología de Artrópodos y Manejo de Plagas. El Colegio de la Frontera Sur (ECOSUR). Carretera Antigua Aeropuerto, Tapachula 30700, Chiapas, México. E-mail: pliedo@ecosur.mx*

Background: Mango (*Mangifera indica* L.) is one of the most widely consumed fruits worldwide due to its nutrient and characteristic flavor and aroma, which are primarily determined by its volatile compounds. The Mexican fruit fly, *Anastrepha ludens*, is considered a species of high economic importance and is classified as a polyphagous pest, as it infests a wide variety of host plants. To develop more effective trapping systems for monitoring and managing fruit flies, it is essential to understand their resource-searching behavior and identify the chemical and visual cues that influence it. The objective of this research was to determine the attraction of *A. ludens* to mixtures of selected volatile compounds emitted by five mango cultivars.

Methods: Field cage tests and electroantennography (EAG) assays were conducted using mass-reared, non-irradiated flies aged 11–14 days. The field cage assays were performed in successive phases. In the first phase, mixtures derived from five cultivars were compared. The mixture of the cultivar that elicited the highest attraction was then selected, and blends containing different numbers of compounds (nine, six, four, three, and binary mixtures) were formulated and evaluated in subsequent phases.

Results: The mixture derived from the Kent cultivar captured significantly more flies than the other treatments. Two mixtures formulated from compounds present in the Kent blend were found to be as attractive as the complete mixture. The mixture of the three major compounds (myrcene, 3-carene, and β -caryophyllene) offers a promising alternative for developing a more effective attractant for *Anastrepha ludens*. Electroantennography (EAG) assays confirmed that both females and males exhibited the highest antennal responses to the same treatments: the Kent mixture, the blend of the three major compounds, the mixture lacking 3-carene, and the blend of the four minor compounds.

Conclusion: Our results indicate that although neither the Kent volatiles blend nor the mixture of the three major compounds can match Ceratrap in attraction efficacy, these mixtures show promise for the development of a semiochemical-based attractant for *A. ludens*.

Key words: Tephritids, Mexican fruit fly, synthetic mixtures, kairomones.

MASS REARING, QUALITY CONTROL, AND STERILIZATION OF DROSOPHILA SUZUKII IN THE SANTA ROSA BIOPLANT (ISCAMEN)

Juan Asfennato, Estela Tur, Rolando Martinez, Asfennato Sol, Jaquelina Tur, José Orts

Institute of Agricultural Health and Quality of Mendoza (ISCAMEN), Mendoza, Argentina.
E-mail: aleasfennato@yahoo.com.ar

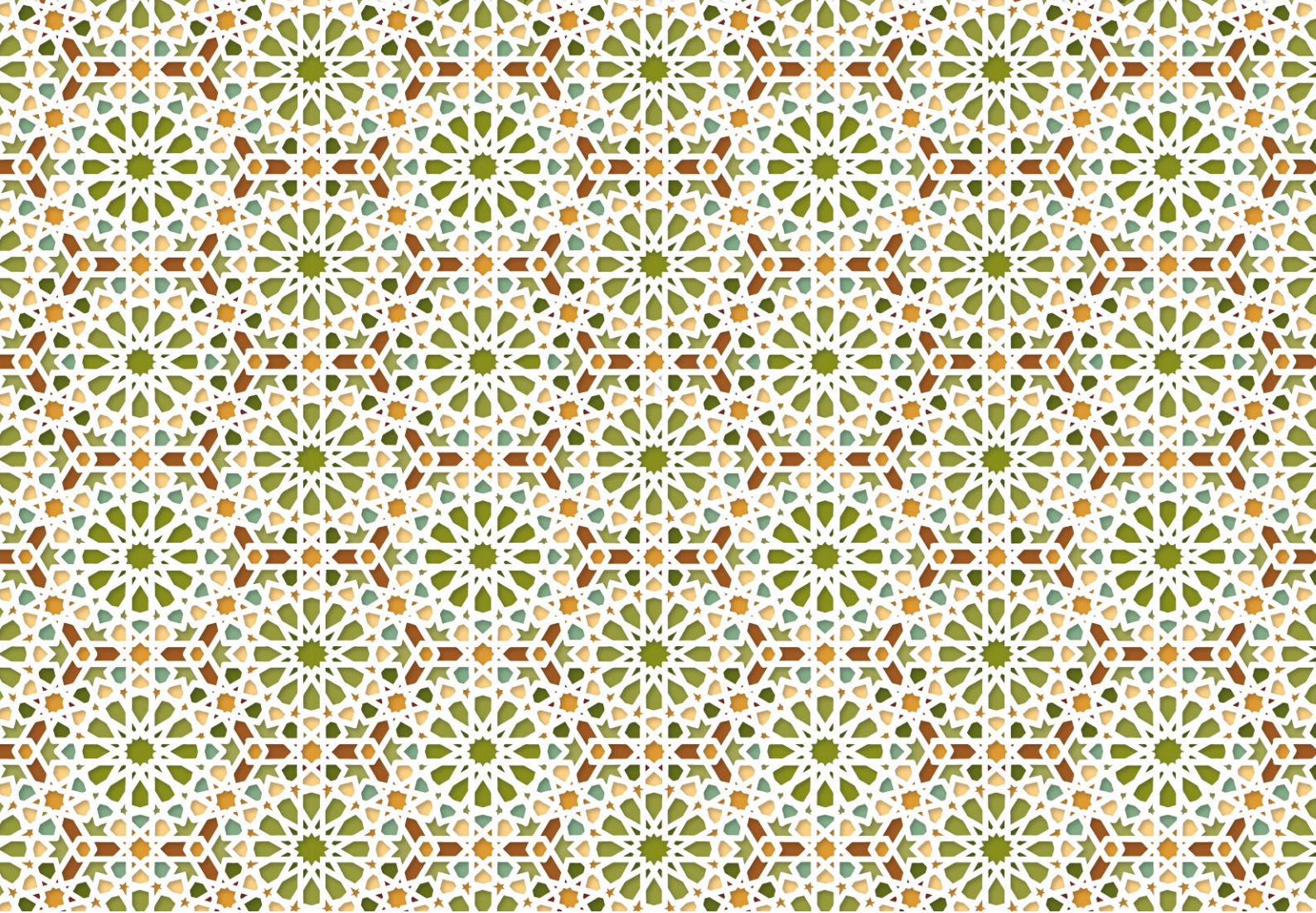
Background: A bisexual colony of *Drosophila suzukii* with 85 generations of inbreeding is currently maintained and structured into two management levels: a filter colony focused on genetic and morphological selection, and an amplification colony dedicated to mass production. The objective is to sustain production and quality parameters compatible with sterile insect production and field application programs.

Methods: The filter colony operates with two Plexiglas cages containing 30 cc of pupae (approximately 6,000 individuals), where manual selection under stereomicroscope is conducted to confirm species identity, body size, and morphological integrity. Egg collection is performed through direct exposure of larval diet to adults for 24 hours, followed by incubation at 22 °C for 7–9 days under controlled humidity (75–85%). Larvae are recovered using the standard larval diet dissolution method, pupae are separated, dried on absorbent paper, and transferred to the amplification colony. The production colony uses 1DS-MAS cages with a capacity of 250 cc of pupae per cycle and a total production of 1,250 cc. The DS-2 larval diet formulation includes sugar, torula yeast, carrot powder, dehydrated potato puree, soybean flour, preservatives, acidifiers, and water. Sterilization is performed using the IMCO-20 irradiator at Santa Rosa Bioplant, applying a total dose of 220 Gy delivered in two passes to ensure homogeneous irradiation.

Results: Average weekly production reaches approximately 100 cc of pupae, equivalent to an estimated 1 million pupae per week. Quality parameters include a mean pupal weight of 1.68 mg, egg viability of 85–95%, adult emergence of 72.5%, proportion of fliers of 71.1%, sterility levels ranging from 95.5% to 99.5%, approximately 220,000 pupae per liter, and an estimated yield of 40 million pupae per ton of diet. Quality parameters have remained stable; however, adult emergence is the most environmentally sensitive parameter, particularly under low relative humidity. Weekly shipments of sterile pupae to Patagonia have been conducted, with transport times of 24–30 hours from irradiation to box opening at destination. Preparations for shipments to Chile are ongoing pending regulatory authorization.

Conclusions: The mass-rearing, selection, production, and sterilization system has been optimized and standardized, allowing the maintenance of stable and reproducible production and quality parameters and ensuring a reliable supply of sterile pupae for logistical evaluation and field applications. The refinement of the rearing protocol supports scalable production according to operational demand, providing flexibility for future program expansion. Humidity control during larval and pupal stages was identified as a critical factor for improving adult emergence.

Key words: mass rearing; *Drosophila suzukii*; Sterile Insect Technique; quality control; irradiation; pupal production;



ORAL COMMUNICATIONS

Genetics and Biotechnology

Integrative Genomic and Ecological Insights into Invasion History, Climate-Driven Adaptation, and Future Biosecurity Risk of the Destructive Fruit Fly *Bactrocera correcta*

Weisong Li^{1,2}, Yuange Duan³, Yujia Qin^{1,2}, Yue Zhang⁴, Yingxuan Li¹, Zuxing Liao¹, Yuan Zhang¹, Tengda Guo¹, Neng Zhang^{1,2}, Sandeep Singh⁵, Nguyen Thi Thanh Hien⁶, Nahallage Dona Asha Dilrukshi Wijegunawardana⁷, Suk Ling Wee⁸, Pablo Deschepper⁹, Marc De Meyer¹⁰, Massimiliano Virgilio¹⁰, Jiajiao Wu¹¹, Shaokun Guo^{1,2}, Lijun Liu^{1,2}, Zihua Zhao^{1,2}, **Zhihong Li**^{1,2,*}

1 State Key Laboratory of Agricultural and Forestry Biosecurity, MARA Key Laboratory of Surveillance and Management for Plant Quarantine Pests, College of Plant Protection, China Agricultural University, Beijing 100193, China. 2 Sanya Institute of China Agricultural University, Sanya 572025, China. 3 Department of Entomology and MOA Key Lab of Pest Monitoring and Green Management, College of Plant Protection, China Agricultural University, Beijing 100193, China. 4 Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China. 5 Department of Fruit Science, Punjab Agricultural University, Ludhiana, Punjab, India. 6 Department of Entomology and Nematology, Plant Protection Research Institute, Vietnam Academy of Agricultural Science, No8 Vien street, Dong ngac, Ha noi, Viet Nam. 7 Department of Bioprocess Technology, Faculty of Technology, Rajarata University of Sri Lanka, Mihintale, Sri Lanka. 8 Centre for Insect Systematics, Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor Darul Ehsan, Malaysia. 9 Royal Belgian Institute for Natural Sciences (RBINS), Biology Department, Vautierstreet 29, 1000 Brussels, Belgium. 10 Royal Museum for Central Africa, Invertebrates Section and JEMU, Tervuren B3080, Belgium. 11 Guangzhou Customs District Technology Center, Guangzhou, 510623, China. E-mail: lizh@cau.edu.cn

Background: The guava fruit fly, *Bactrocera correcta*, is a highly destructive pest posing a significant threat to global biosecurity. Despite its economic impact, critical knowledge gaps remain regarding its precise geographic origin, historical invasion routes, and the specific genomic mechanisms that allow it to adapt to diverse climates. Understanding these factors is essential for developing effective quarantine and management strategies.

Methods: We conducted a comprehensive analysis using genome-wide single nucleotide polymorphisms (SNPs) across the species' global distribution. Our approach integrated comparative genomics, genome–environment association studies (GEAS), and RNA interference (RNAi) to validate gene function. Furthermore, we employed ensemble species distribution modeling and a hierarchical random forest model to investigate environmental adaptation and forecast future invasion risks on a global scale.

Results: The species was traced back to an origin in southern South Asia, identifying two distinct invasion routes into China via Yunnan and Hainan Provinces. Genomic analysis revealed that the expansion of the *Hsp70* gene family facilitates thermal tolerance, while the adaptive evolution of *Cyp4s3* drives cold adaptation; silencing these genes via RNAi significantly decreased survival rates under extreme temperatures. Global modeling identified northern Australia, central-southern Africa, and central-southern America as high-risk regions for future outbreaks.

Conclusions: This study uncovers the genomic architecture underlying the high environmental plasticity of *B. correcta*. By identifying key genes responsible for temperature resistance and mapping high-risk geographic zones, we provide a multifaceted predictive framework. This framework is instrumental for biosecurity agencies to assess and mitigate the expansion of invasive pests more effectively.

Key words: *Bactrocera correcta*, genome, invasion history, thermal adaptation

CRISPR/Cas9-based *white pupae* mutant lines in *Bactrocera dorsalis*, *Bactrocera zonata* and *Zeugodacus tau* for sterile insect technique applications

**Chrysanthi Ioannidou^{1,2}, Maria-Eleni Gregoriou¹, Zhi Gong³, Marc F. Schetelig^{4,5},
Elena Drosopoulou², Kostas Bourtzis¹**

¹Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna, Austria.

²Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece.

³Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Hainan, China.

⁴Department of Insect Biotechnology in Plant Protection, Institute for Insect Biotechnology, Justus-Liebig-University Gießen, Gießen, Germany.

⁵Liebig Centre for Agroecology and Climate Impact Research, International Atomic Energy Agency Collaborating Centre, Justus-Liebig-University Gießen, Gießen, Germany. E-mail: C.Ioannidou@iaea.org

Background: *Bactrocera dorsalis*, *Bactrocera zonata*, and *Zeugodacus tau* are recognised as extremely invasive insect species, making them high priority for pest control strategies, including the sterile insect technique (SIT), which depends on the mass production and release of sterilized males. Male-only releases, enabled by genetic sexing strains (GSS), can improve the efficiency and cost-effectiveness of SIT. In this study, using a neoclassical genome-editing approach that leverages targeted CRISPR-induced marker mutations followed by sex-specific genetic rescue, we evaluate the *white pupae* (*wp*) gene as a selectable marker for GSS development in the three species.

Methods: Orthologs of the *wp* gene were identified by comparative genomic analysis and targeted using CRISPR/Cas9-mediated mutagenesis in all three species. Resulting edits were screened to establish homozygous *white pupae* mutant lines, which were subsequently phenotypically characterized.

Results: CRISPR/Cas9-mediated disruption of exon 3 of the *wp* gene generated multiple independent knock-out lines exhibiting the white pupae phenotype in all three species with distinct mutation events identified in all three species *B. dorsalis*, *B. zonata*, and *Z. tau*. In addition to white pupal coloration, mutant *B. zonata* individuals exhibited partial depigmentation of the thorax and abdominal tergite, while mutant *Z. tau* individuals showed reduced apical wing spots.

Conclusions: CRISPR/Cas9 disruption of the conserved *wp* gene successfully generated homozygous mutant lines in *B. dorsalis*, *B. zonata*, and *Z. tau* demonstrating that *wp* is a robust and transferable selectable marker for genetic sexing strain development using the CRISPR/Cas9-based neoclassical approach.

Key words: tephritids, insect pest control, genetic sexing strains, genome editing

Engineering and Expression of Y-Linked Genetic Markers in *Ceratitis capitata*

Cristina Borghesi^{1,2}, Serafima Davydova³, Roswitha A. Aumann^{1,2}, Angela Meccariello³ & Marc F. Schetelig^{1,2}

¹ Department of Insect Biotechnology in Plant Protection, Justus Liebig University Giessen, Giessen, Germany;

² Liebig Centre for Agroecology and Climate Impact Research, International Atomic Energy Agency Collaborating Centre, Justus Liebig University Giessen, Giessen, Germany;

³ Department of Life Sciences, Imperial College London, London, United Kingdom.

E-Mail: cristina.borghesi@agrar.uni-giessen.de

Background: The Sterile Insect Technique (SIT) is a safe, environmentally friendly, and species-specific method for controlling insect pests. SIT efficiency depends on genetic sexing strains (GSS) that enable sex separation for male-only field releases. Classical GSS rely on chromosomal translocations between an autosome and the Y chromosome to achieve male-specific marker expression. This strategy can negatively affect strain stability and biological performance. Neoclassical GSS based on engineered Y-linked genetic markers represent a promising alternative. However, their development is constrained by two major challenges: achieving reliable integration of genetic constructs into the Y chromosome and ensuring functional expression of selectable markers once integrated. Although the Y chromosome is largely heterochromatic, reduced gene expression cannot be attributed solely to chromatin context, as promoter-dependent effects have been observed.

Methods: We exploited previous reports that transposon-mediated remobilization to the Y chromosome can occur from an X-linked insertion site in *Ceratitis capitata*. A previously established strain in our laboratory carrying an X-linked engineered *white pupae* (*wp*) selectable marker and a fluorescent reporter, driven by distinct promoters, was used to induce transposon remobilization events. A Y-linked insertion was isolated and characterized, and the expression of both markers was subsequently analysed.

Results: We successfully generated a Y-linked insertion of the engineered genetic cassette, demonstrating that integration into the Y chromosome is feasible. In the resulting Y-linked line, the *wp* selectable marker retained functional expression, whereas the fluorescent reporter was not visibly expressed. These contrasting outcomes indicate that successful Y-linked integration does not guarantee marker activity and that gene expression on the Y chromosome is strongly dependent on the regulatory elements and markers employed.

Conclusions: Our findings show that the primary limitation for developing Y-linked genetic sexing tools in *C. capitata* lies not in achieving chromosomal integration, but in obtaining reliable, promoter-compatible expression of genetic markers. The observation that the *wp* marker remains functional on the Y chromosome, while the fluorescent reporter does not, underscores the need for systematic evaluation of promoters and regulatory elements. Approaches such as the use of genetic insulators or alternative promoter architectures may help overcome Y-linked regulatory constraints and will be explored in future work.

Key words: SIT, GSS, Mediterranean Fruit Fly, transposon remobilization, *white pupae*

Telomere-to-Telomere Genome Datasets for Pests as a Foundation for Future Pest Management: A Case Study on *Bactrocera dorsalis*

Wei Liu, Qiang Lin, Peng Cui, Guirong Wang

Shenzhen Branch, Guangdong

Laboratory of Lingnan Modern Agriculture, Key Laboratory of Synthetic Biology, Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China

Background Genomic approaches are increasingly vital for pest control research, enabling the discovery of molecular targets, understanding resistance mechanisms, and developing biological control strategies. High-quality reference genomes are foundational to these efforts. The advent of third-generation sequencing technologies has ushered in the telomere-to-telomere (T2T) era, addressing challenges in assembling highly repetitive and structurally complex regions of the genome.

Methods. In this study, we successfully assembled a high-quality telomere-to-telomere (T2T) genome for *Bactrocera dorsalis*, a representative model for studying dipteran pests. The assembly resolves previously inaccessible regions, including centromeric and pericentromeric regions, sex chromosomes, and structurally complex loci with tandemly duplicated genes, such as odorant receptor gene clusters.

Conclusions These results provide detailed structural insights into repetitive and functionally critical regions of the genome, laying a solid foundation for advancing Dipteran genomics and developing future pest management strategies.

Key words: *Bactrocera dorsalis* , genome assembly ,Telomere-to-Telomere Genome Datasets**Session 3.** Genetics and biotechnology

ENGINEERING AN EARLY DEVELOPMENT SEX SEPARATION TOOLKIT FOR MEXFLY, *ANASTREPHA LUDENS*

Georgia Gouvi¹, Junru Liu², Ting Yang², Nikolai Kandul², Evan Braswell³, Omar Akbari^{2*}, Angela Meccariello^{1*}

¹Department of Life Sciences, Imperial College London, United Kingdom, ²Department of Cell and Developmental Biology, University of California, United States, ³USDA APHIS PPQ Science and Technology Insect Management and Molecular Diagnostic Laboratory, Texas, United States, *Corresponding authors: a.meccariello@imperial.ac.uk; oakbari@ucsd.edu

Background: The Mexican fly, *Anastrepha ludens*, is a major agricultural pest affecting fruit production and industry across the Western Hemisphere. Its widespread distribution has created an urgent need for effective population suppression and invasion prevention strategies. Among these, the Sterile Insect Technique (SIT), mainly supported by the genetic sexing strain (GSS) black pupae, has been extensively implemented. Despite these efforts, Mexfly remains a substantial economic threat to crop production, carrying the major limitation of the current GSS systems, the absence of an early developmental stage sex-sorting mechanism, which reduces operational efficiency and limits technology transfer to other pest species.

Methods: Here, we present the successful application of a novel sex-separation strategy based on a sex-specific intron of the *transformer* (*tra*) gene previously validated in *Ceratitis capitata* (SEPARATOR system). This approach employs both male- and female-specific fluorescent markers, enabling precise sex separation at early developmental stages. The system removes a key bottleneck in mass-rearing workflows by allowing earlier and more efficient sex sorting.

Results: Both male- and female-specific SEPARATOR strains were generated and characterized, demonstrating the expected phenotypic outcomes. These results support the development of improved genetic sexing strains and provide a scalable toolkit for sex separation in non-model tephritid species.

Conclusions: This work establishes a functional early developmental sex-separation system in *Anastrepha ludens*, demonstrating the feasibility of transformer-intron-based sorting in a non-model tephritid. The SEPARATOR platform enhances genetic sexing efficiency and provides a transferable framework for next generation SIT programs.

Keywords: Mexfly, SIT, GSS, Tephritids, sex sorting, transformer intron, SEPARATOR

CONDITIONAL SEXING SYSTEMS FOR SIT BASED ON SYNTHETIC BIOLOGY APPROACHES

Kaatje Knüwer¹, Hassan M. M. Ahmed^{1,2} & Ernst A. Wimmer¹

¹Department of Developmental Biology, Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Georg-August-University Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany. ² Department of Crop Protection, Faculty of Agriculture – University of Khartoum, 13314 Khartoum North, Sudan.
E-mail: kaatje.knuewer@stud.uni-goettingen.de

Background: The Mediterranean fruit fly (*Ceratitidis capitata*, Wiedemann) is one of the most devastating pest insects worldwide and is established across Africa, Southern Europe, Australia, and parts of America. One of the most effective management techniques is the sterile insect technique (SIT), which relies on the large-scale release of sterilized males. The Vienna 8 genetic sexing strain is currently the most widely used system to separate males from females through a combination of heat-shock-induced female lethality and pupal colour selection. However, the required heat-shock treatments pose an extra step in rearing the flies, and the partial aneuploidy of the current translocation-based strain can reduce male offspring numbers. Consequently, the development of new genetic sexing strains (GSS) should enable more cost-effective mass-rearing for SIT programs.

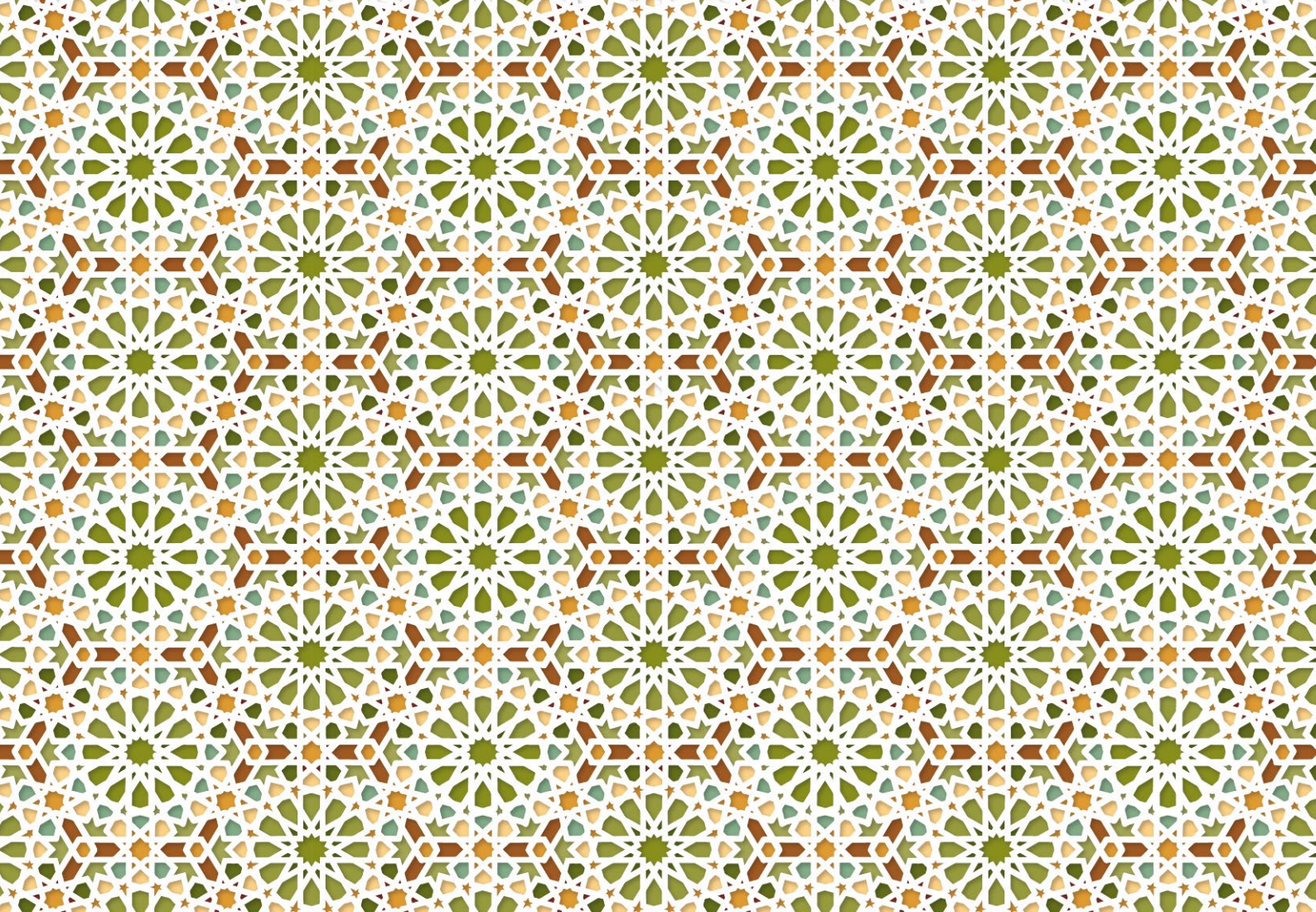
Methods: In recent years, novel male-determining factors have been discovered in several pest species, including the *Maleness-on-the-Y* (*MoY*) locus in *C. capitata*. Building on this, we generated several fly strains that conditionally overexpress the male-determining factor during early developmental stages. The overexpression is genetically linked to fluorescent markers and can be controlled by food-supplemented effector compounds such as tetracycline, allowing regulated expression.

Results: Conditional overexpression of the male-determining factor during embryogenesis resulted in only male offspring, which was reversible by tetracycline food supplementation, leading to a 0.6 ratio of male-to-female offspring. Preliminary data indicate that embryonic overexpression is more effective than overexpression during oogenesis, consistent with the natural developmental expression pattern of the *MoY*-locus in *Ceratitidis capitata*. Furthermore, stable *MoY* overexpression during embryogenesis proved more efficient in inducing exclusively male offspring than RNA interference targeting *transformer*.

Conclusions: The developed genetic sexing strains have the potential to simplify the production pathway for male-only releases by removing the need for heat-shock treatments. Our approach could also increase the number of males produced from a given number of eggs through the sex conversion of XX-embryos to males. However, direct comparative production tests against the Vienna 8 strain will be required to confirm this. Furthermore, sexing systems based on male-determining factors may be adaptable to other pest species targeted by SIT programmes.

Key words: *Ceratitidis capitata*, Mediterranean fruit fly, sterile insect technique, synthetic biology, sexing system, genetic sexing strain

~~Session 3: Genetics and Biotechnology~~



ORAL COMMUNICATIONS

Chemical Ecology and Attractants

OPTIMAL LOCATION AND DISTRIBUTION OF HYDROLYSED PROTEIN IN A TREE CANOPY FOR CONTROL OF *CERATITIS CAPITATA*, *C. COSYRA* AND *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

Christopher Weldon¹, Msizi Ramaoka¹ and Aruna Manrakhan^{2,3}

¹Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Hatfield 0028, Pretoria, South Africa

²Citrus Research International, Nelspruit 1200, South Africa

³Department of Conservation Ecology and Entomology, Stellenbosch University, Matieland 7602, Stellenbosch, South Africa

Email: cwweldon@zoology.up.ac.za

Background: Hydrolysed protein is the attractant of choice for use in bait sprays or stations for fruit fly control. Understanding how different species respond to hydrolysed protein, and its best location and distribution is needed to optimise fruit fly control. We determined the responses of *Ceratitis capitata*, *C. cosyra*, and *Bactrocera dorsalis*, to hydrolysed protein in a tree canopy.

Methods: To do so, we first recorded the response of each species to baits placed at four heights in the canopy (ground, lower, mid-, and upper canopy) with regard to fly age (one- or 10-day-old), sex, and diet (protein-deprived or fed). Secondly, we evaluated the response by females of each species to baits applied at two densities: 10 droplets of 200 µL protein hydrolysate solution (high bait density) or a single drop of 2000 µL (low bait density).

Results: In both experiments, *C. capitata* responded more than the other species to baits. Response by all three species was highest to baits in the mid- to upper canopy. One-day-old protein-fed flies were least responsive, while 10-day-old protein-deprived flies were most responsive. Protein-deprived females responded to baits more than males with the same diet. Response was greatest at high bait density. When protein-fed, very few *B. dorsalis* or *C. cosyra* responded when bait density was low, but *C. capitata* still responded.

Conclusions: Our results show that protein hydrolysate baits need to be in a tree canopy and evenly distributed in a tree to ensure fruit fly response, particularly when multiple species are being targeted. Since bacteria are an important natural source of protein for fruit flies, protein baits may be less effective in warm and humid regions that support microbial growth or in orchards where sanitation is poorly implemented.

Key words: Bait location, bait spray, bait station, diet, hydrolysed protein

Conservation of male attractant detection across Tephritidae

Vincent Jacob¹, Emma Persyn^{1,2}, Nicolas Slomiany¹ & Emmanuelle Jacquin-Joly²

¹ CIRAD, Université de la Réunion, UMR PVBMT, 7, ch. de l'IRAT, F-97410 Saint-Pierre, La Réunion, France

² INRAE, Sorbonne Université, CNRS, IRD, UPEC, Université Paris Cité, Institute of Ecology & Environmental Sciences of Paris, Route de Saint-Cyr, F-78026 Versailles Cedex,

E-mail: vincent.jacob@cirad.fr

Background: Male attractants are widely used to detect and control Tephritid pest species. When naturally ingested by males, these compounds modify the sex pheromone composition, enhancing male attractiveness to females and thereby increasing individual reproductive success. Over evolutionary time, it is believed that sexual selection emerged, and the chemical identity of these attractants underwent multiple shifts. Yet it remains unclear whether these changes were driven primarily by differences in the sensory detection of the compounds or by decision-making processes that govern behavioural responses.

Methods: Behavioural and electrophysiological responses to compounds known to attract males in at least one Tephritid species were compared in males from nine species: *Bactrocera dorsalis*, *Bactrocera zonata*, *Ceratitis capitata*, *Ceratitis catovii*, *Ceratitis quilicii*, *Dacus ciliatus*, *Dacus demmerezi*, *Neoceratitis cyanescens*, and *Zeugodacus cucurbitae*. Olfactory attraction was assessed using the high-throughput Flywalk behavioural assay, and olfactory detection was measured with three-point electroantennography and electropalpography.

Results: Behaviourally, species fell into three groups: methyl eugenol responders, cue lure responders, and non-responders, with small variations within groups. Most compounds elicited peripheral responses in males across all species, with only weak connection to behavioural preferences. Consistently, peripheral responses did not predict behavioural groups. Notably, among the two species attracted to methyl eugenol, only *B. zonata* showed a stronger antennal response to this compound than other species, whereas cue lure elicited weak peripheral responses in all species.

Conclusions: Our findings suggest that male attraction in Tephritidae may have evolved through pre-existing signal reinterpretation in the central nervous system, leading to novel behavioural outputs. Overall, this study provides valuable behavioural and electrophysiological data for understanding olfactory mechanisms underlying responses to semiochemicals used in pest management.

Key words: Chemical ecology, male lure, Electroantennography, Electropalpography, Flywalk

Mating competitiveness and compatibility of genetically engineered strains of Queensland fruit fly for sterile insect technique

Md Forhad Hossain^{1,2*}, Most Mottakina Akter¹, Md. Jamil Hossain Biswas¹, Mst Raosonara Khatun¹, Rajendra Regmi¹, Tien Hung Nguyen¹, Masuma Akter³, Fozia Masood¹, Afrin Priya Talukder¹, Vivian Mendez¹, Peter Crisp⁴ and Phillip W. Taylor¹

¹Applied BioSciences, Macquarie University, Sydney, NSW, Australia. ²Insect Biotechnology Division, IFRB, AERE, Bangladesh Atomic Energy Commission, Bangladesh. ³School of Natural Sciences, Macquarie University, Sydney, NSW, Australia. ⁴South Australian Research and Development Institute, Adelaide, SA, Australia. E-mail: mdforhad.hossain@hdr.mq.edu.au

Background: Queensland fruit fly (Q-fly) (*Bactrocera tryoni*), is the most damaging insect pest of horticulture in Australia. The sterile insect technique (SIT) provides an effective, species-specific, and environmentally benign method for regional management of Q-fly outbreaks. Genetically engineered strains have recently been developed to enable male-only releases through sorting by sex differences in pupal colour (Grey-Scale Exon-3 [GS Exon-3], Genetic Sexing Strain [GSS]) and to enable phenotypic marking through yellow body colour (Yellow-1, Yellow-2).

Methods: The present study assesses field cage mating competitiveness and mating compatibility, and routine quality control parameters, of these engineered strains, comparing them with the unengineered laboratory strain that is currently used in SIT programs.

Results: GS Exon-3, GSS, and Yellow-1 strains were comparable to the unengineered strain in mating competitiveness, mating compatibility, copula latency, copula duration, and indices of sexual isolation when paired with both wild and laboratory flies, as well as for emergence, flight ability, and egg hatch rate for all except GSS, which had reduced egg hatch rate. However, Yellow-2 had significantly lower mating performance, percentage of emergence, flight ability, and hatch rate, indicating poor suitability for SIT programs.

Conclusions: These results support continued investigation of GSS and Yellow-1 as promising candidates for deployment in SIT programs, offering potential improvements in cost-effectiveness, operational safety, and efficiency through male-only release or phenotypic marking.

Key words: *Bactrocera tryoni*, sterile insect technique, Genetic sexing strain, Mating competitiveness, Mating compatibility

ABSTRACT

Cuticular hydrocarbon communication in *Bactrocera*: a novel tool for enhanced pest control strategies.

Antonio Pompeiano¹, Francesca Scolari², Pavlína Kyjaková³ & Lucie Vaníčková¹

¹ Department of Forest Botany, Dendrology and Geobiocoenology, Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1/1665, 613 00 Brno, Czech Republic. E-mail: antonio.pompeiano@mendelu.cz

² Institute of Genetics and Biomedical Research, National Research Council, Italy

³ Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences

Background: Chemical communication, particularly through cuticular hydrocarbons (CHs), is a key mediator of reproductive behavior in *Bactrocera* species. These compounds vary with sex, age, physiological condition, and environmental factors, and offer untapped potential for enhancing biologically based pest management strategies such as the Sterile Insect Technique (SIT) and the Male Annihilation Technique (MAT). This research aims to deepen understanding of CH profiles in *Bactrocera* spp., exploring their role in intra- and interspecific interactions. Emphasis is placed on how various intrinsic and extrinsic factors influence CH composition, with the goal of identifying reliable markers of sexual maturity and fitness in target species.

Methods: Using advanced analytical techniques such as comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-MS), we are developing and optimizing protocols to characterize CHs in several economically important species, beginning with *B. oleae*. Complementary statistical tools, including principal component analysis (PCA) and clustering methods, are employed to interpret the complex chemical data.

Results: GC×GC-MS analysis revealed complex CH profiles in *Bactrocera* spp., with clear quantitative differences associated with sex and age in *B. oleae*. Multivariate analyses (PCA and clustering) consistently discriminated immature and sexually mature individuals and separated males and females based on relative abundance patterns of long-chain and methyl-branched hydrocarbons. Preliminary comparisons further indicated species-specific CH signatures and detectable effects of physiological and rearing conditions on CH composition. These results demonstrate that CH profiles capture biologically meaningful variation relevant to sexual maturity, fitness, and species recognition.

Conclusions: The broader objective of this work is to apply insights from CH variation toward improving pest control tactics—particularly by refining sterile male quality assessment and enhancing communication-based trapping or mating disruption approaches. While still at an early stage, the project supports the long-term development of environmentally sustainable, species-specific control strategies grounded in chemical ecology.

Key words: *Bactrocera*, cuticular hydrocarbons, enhanced pest control, GC×GC/MS,

Session: ~~Chemical Ecology and Attractants~~

PERFORMANCE OF DIFFERENT HOMEMADE TRAPS USING THE ORGANIC FOOD BAIT MANGO PROTECT AGAINST FRUIT FLIES IN BURKINA FASO

Karim Nèbié¹, Habibata Semdé², Céline Bonkounougou¹, Issaka Zida¹, Boureima Tassembédo¹, Evelyne Compaoré¹, Julienne N. Gué¹, Boris Compaoré³, Modeste Florentin Bationo³, Jean-François Houmard³, Pascal Waelti⁴, Rémy A. Dabiré¹

¹Centre National de la Recherche Scientifique et Technologique, Institut de l'Environnement et de Recherches Agricoles

²Université Nazi BONI, Institut du Développement Rural

³Centre Ecologique Albert Schweitzer

⁴Haute Ecole Spécialisée Bernoise

Corresponding author: nebkar87@gmail.com

Background: In Burkina Faso, fruit flies are a major constraint on the production of fruits and vegetables. An organic food bait called Mango Protect has been developed to control these pests. However, the lack of an accessible trap for its use hinders its adoption by producers. The study aimed to evaluate the performance of different homemade traps in capturing fruit flies in mango orchards.

Methods: Three groups of traps were designed and tested in comparison to the McPhail trap. These include the group of short cylindrical traps (CyS) A and B, the group of long cylindrical traps (CyL) A and B, and the group of bottle traps (PLB) A and B. The traps were installed in four mango orchards located in the localities of Sipigui, Kourinion, Diéri, and Orodara. In each orchard, the traps were installed in a randomized line with 5 repetitions. Each trap contained 400 ml of Mango Protect. Trap checks and product renewal were carried out every ten (10) days for 90 days (June 20 - September 15, 2025).

Results: Nine fruit fly species belonging to three genera (*Bactrocera*, *Ceratitis*, *Zeugodacus*) were captured with predominance of *Bactrocera dorsalis* (95-99% of captures). At all study sites, the highest FTD were recorded with McPhail traps and those of the PLB group. Indeed, captures ranged from 4 to 126.52 flies/trap/day depending on the sampling dates at the Orodara site. At the Kourinion site, captures ranged from 1 to 34.86 flies/trap/day depending on the sampling dates. In Diéri, captures ranged from 4 to 135.76 flies/trap/day depending on the sampling dates. In Sipigui, an average of 2 to 117.18 flies/trap/day was recorded. Traps of the CyS and CyL groups recorded average captures ranging from 1 to 67.34 flies/trap/day as function of sites and observation frequencies. Considering an overall analysis across all sites, the traps of the PLB group and McPhail recorded an average of 4.44 to 60.09 flies/trap/day and 5.81 to 86.23 flies/trap/day, respectively. No significant differences observed between the PLB group traps and the conventional McPhail trap.

Conclusions: The homemade traps from the PLB group perform almost as well as the conventional McPhail trap. Considering their physical and financial accessibility compared to the McPhail trap, we recommend that producers use the PLB-A trap with the Mango Protect bait to control fruit flies.

Key words: *Bactrocera dorsalis*, *Ceratitis cosyra*, food attractant, organic production.

Improving Male Annihilation Technique Against Cuelure Responders: A Field Test with Individual-Based Tracking

Nicholas C. Manoukis, Charlotte Aldebron, Matthew S. Siderhurst & Corinna Bazelet

United States Department of Agriculture – Agricultural Research Service, European Biological Control Laboratory, 810 avenue du campus Agropolis, Montferrier-sur-Lez 34980, FRANCE. E-mail: nicholas.manoukis@usda.gov

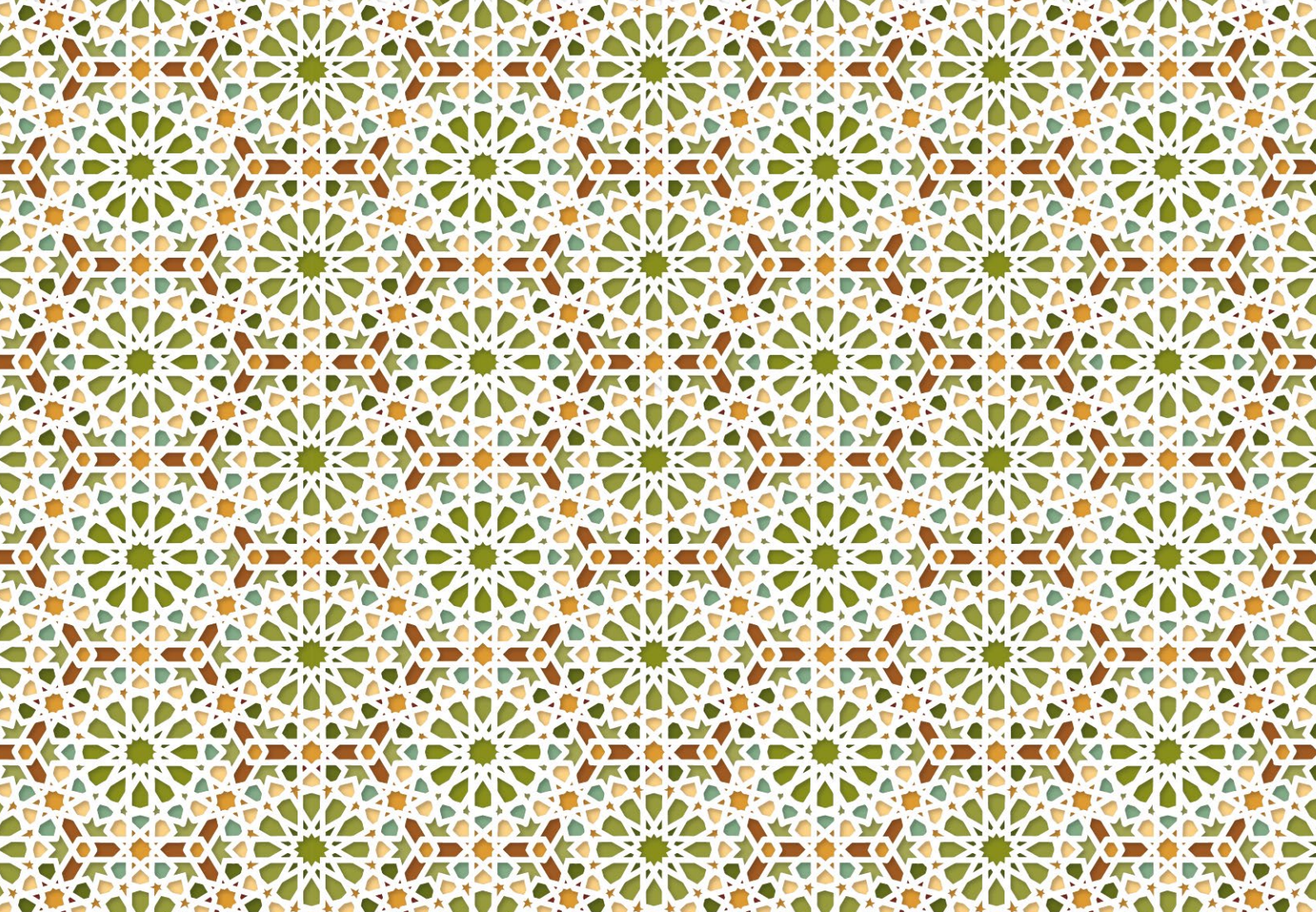
Background: Male annihilation technique (MAT) is a well-known tool against some species of tephritid fruit flies, namely those for which a powerful male attractant is known. MAT is the application of many spots per unit area containing the male lure and a toxicant, to attract and kill males. It has been shown to be effective especially against methyl-eugenol (ME) responding flies, to the point of eliminating populations. Work in the last years has shown that for ME responders MAT can be improved by decreasing the application density from the previous recommendation of about 230 spots/km² to around 110 spots/km². MAT is more rarely used against cuelure (CL) responders, as it is thought to be less effective - mainly because CL is less attractive to males of those species. Historically, a very high application density is recommended. We set out to test if reducing the application density of CL male annihilation technique (CL-MAT) would improve its efficacy, as has been seen with ME.

Methods: We assessed the percentage of *Zeugodacus cucurbitae* males killed via mark-release-recapture (MRR) experiments at varying application densities from 10 to 100% of the general recommendation (600 spots/km²), with fresh and two-week old applications. We used two products for CL-MAT, one in a fiber-board form and a formulation employing a waxy matrix. We also tracked individual males fitted with harmonic radar (HR) tracking devices, enabling us to test the hypothesis that the decreased effectiveness of CL-MAT at higher densities is driven by interference and decreased ability to locate point sources of the lure.

Results: We found that at the usual application density CL-Mat was ineffective, killing about 10% of the released males over three days. The kill rate increased significantly, to almost 40%, at the lowest application density we tested with aged lures. Kill was higher with aged rather than fresh applications. We did not measure any difference in effectiveness between the products tested, though this comparison was limited. Our measurements of movement via HR tracking showed that it is reduced in plots that had a higher application density, supporting the idea that saturation with male lures is leading to arrestment and decreased contact with toxicant.

Conclusions: This study suggests that CL-MAT may be very effective against species that respond to cuelure, such as *Z. cucurbitae*. However, the most effective application density is about 1/10th the usual recommendation, or perhaps lower. Tracking results and increased effectiveness of aged applications both support the hypothesis that over-application causes saturation, hindering the ability of individual males to find point sources. Future work on other species that respond to cuelure would be valuable to confirm these results.

Key words: Harmonic radar, male attraction technique, melon fly, eradication



ORAL COMMUNICATIONS

Risk Assessment, Quarantine and Post-harvest

The Oriental fruit fly (Diptera: Tephritidae) at its southern adventive range limit: ecology and development of control options

Aruna Manrakhan^{1,2}, John-Henry Daneel¹ & Leani Serfontein¹

¹Citrus Research International, Mbombela, South Africa

²Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa

E-mail: aruna@cri.co.za

Background: *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), Oriental fruit fly, is an invasive pest of commercial fruit that was declared established in the northern parts of South Africa in 2013. To date, the distribution of the pest within the country remains restricted. Studies were carried out to track the abundance and population dynamics of *B. dorsalis* in Mpumalanga Province, in the north of South Africa. Additionally, efficacy of pre-harvest and post-harvest measures for control of the pest were quantified.

Methods: Population dynamics of *B. dorsalis* were monitored at least once a month using methyl-eugenol baited traps from October 2020 to October 2022 at three sites in Mpumalanga Province, with altitudes ranging from 452 to 1 741m above sea level. Pre-harvest control measures were assessed by quantifying the toxicity of three protein-based bait stations (M3 bait station, Cera Trap and Magnet Med, and a protein bait spray GF-120) against laboratory reared *B. dorsalis* in field cages. The toxicity of bait stations was evaluated at various times following aging in the field: fresh, 4 weeks, 8 weeks and 12 weeks. Post-harvest control was assessed by determining the efficacy of cold treatment on *B. dorsalis* in fruit and comparing it to *Ceratitidis capitata* (Wiedemann). The efficacy of an internationally approved treatment schedule for *C. capitata* (of 2°C for 18 days) was thereafter confirmed in large scale trials on *B. dorsalis*.

Results: In the north of South Africa, *B. dorsalis* exhibited single population peaks. The pest had a higher prevalence at the low-altitude site in comparison to the sites at higher altitudes. At the highest altitude site, there were periods of up to six months after onset of winter with no catches. *Bactrocera dorsalis* catches were significantly influenced by temperature, increasing with rising temperatures. High mortality levels of *B. dorsalis* (above 60%) were recorded following exposure to tested baits. The type of bait and bait age influenced fly mortality. *Bactrocera dorsalis* was more cold susceptible than *C. capitata*. The cold treatment schedule of 2°C for 18 days was effective in killing *B. dorsalis* larvae at the Probit 9 level.

Conclusions: These findings indicate that *B. dorsalis* populations at the southern limit of the species' adventive range are less pronounced than those reported from other parts of Africa where the pest persists at higher densities throughout much of the year. Currently registered protein-based baits in South Africa are effective for pre-harvest suppression of the pest, and post-harvest cold treatments used for *C. capitata* provide effective control of *B. dorsalis*, supporting their use in phytosanitary systems.

Keywords: *Bactrocera dorsalis*, cold treatment, ecology, protein baits

RAPID ORIGIN ASSIGNMENT OF *BACTROCERA DORSALIS* USING NUCLEAR SNPS OR COI BARCODES

Wannes Dermauw¹, Sam Vanbergen^{2,3}, Lore Esselens², Fanny Kratz², Pablo Deschepper⁴, Jochem Bonte¹, Marc De Meyer² & Massimiliano Virgilio²

¹Flanders Research Institute for Agriculture, Fisheries and Food, Burgemeester Van Gansberghelaan 96, 9820 Merelbeke-Melle, Belgium; ²Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren, Belgium; ³KU Leuven, Charles Deberiotstraat 32 - box 2439, 3000 Leuven, Belgium; ⁴Royal Belgian Institute for Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium. E-mail: wannes.dermauw@ilvo.vlaanderen.be

Background: The oriental fruit fly, *Bactrocera dorsalis*, is a pest of several commercial fruit crops and listed as a priority quarantine pest within the European Union (EU). Between 2023–2025, adult males were detected for the first time in Belgium through national surveillance at public markets and community gardens, prompting an investigation into their geographic origins and whether detections reflected a single introduction or multiple incursions. Historic Belgian interceptions of larvae in imported fruit—particularly mangoes—further motivated origin tracing to guide pathway management and rapid response.

Methods: Trapped adult males were identified as *B. dorsalis* morphologically, whereas intercepted larvae were identified using molecular assays, supported by their morphology. All specimens were subjected to whole-genome sequencing (WGS). Nuclear genetic variation was compared against broad reference datasets to infer geographic origin. In parallel, a phylogenetic analysis of mitochondrial genomes was performed to evaluate concordance with assignments based on nuclear variation.

Results: Analyses of nuclear single nucleotide polymorphism (SNP) variation and mitochondrial genome phylogeny provided contextual evidence for differentiation between African and Asian reference populations. Within this framework, the majority of trapped adults were associated with Asian populations, whereas almost all intercepted larvae clustered with African reference material. Formal origin assignment was achieved using a reduced diagnostic nuclear SNP panel and a standardised discriminant analysis of principal components (DAPC) of COI barcodes. These approaches were largely concordant, while revealing occasional mitonuclear discordance.

Conclusions: Genetic analyses revealed that the *B. dorsalis* adult males trapped in Belgium represent multiple, independent incursions. The difference in origin between trapped adults and larvae found in imported fruit, suggests that *B. dorsalis* adult males likely entered via intra-EU trade or unauthorized fruit imports. The diagnostic nuclear marker panel and DAPC of COI barcodes are practical, scalable tools for national plant protection agencies and reference laboratories, enabling faster and more confident origin tracing, pathway mitigation, and targeted phytosanitary measures.

Key words: origin tracing, oriental fruit fly, *Bactrocera dorsalis*, pathway analysis

Session 5 - Risk Assessment, Quarantine and Post-harvest

Sperm in traps: assessment of female mating status to increase monitoring efficiency

Diana Pérez-Staples¹, Gisela Castillo², Solana Abraham², Betzabé Verónica-Murrieta¹, Abraham Moreno-Mejía¹, Aldebron Charlotte³, Kamala Jayanthi⁴, Jaipal S. Choudhary⁵, Nicholas Manoukis^{3,6}, Laura Moquet⁷, Corinna Bazelete⁸

¹INBIOTECA, Universidad Veracruzana, Xalapa, Veracruz, Mexico. Email: diperez@uv.mx ²Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales (LIEMEN), PROIMI-Biotecnología, CONICET, Tucumán, Argentina. ³USDA, Daniel K. Inouye US Pacific Basin Agricultural Research Center, Hilo, Hawaii. ⁴Indian Institute of Horticulture Research, Bangalore, Karnataka, India. ⁵ICAR RCER, Farming System Research Centre for Hill and Plateau Region, Plandu Ranchi, Jharkhand-834010, India. ⁶USDA Agricultural Research Service, European Biological Control Laboratory (EBCL), 810 avenue du Campus Agropolis 34980 Montferrier-sur-Lez, France. ⁷UMR PVBMT, CIRAD, Saint-Pierre, La Réunion, France. ⁸USDA APHIS PPQ Science and Technology Venture II, 920 Main Campus Drive, Cube 5A-15 Raleigh, NC 27606.

Background: The success of monitoring pest species is key to influencing mitigation decisions and management actions. Accurate determination of mating status has economic implications because enacting quarantines is expensive, as incorrect assessment can lead to costly and ineffective quarantine actions. Here, we determined the likelihood of correctly determining mating status in pestiferous tephritid fruit flies from traps, and how different baits and dyes could impact the correct assessment.

Methods: Mated and unmated *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha fraterculus*, *Ceratitis capitata*, *Bactrocera dorsalis*, *Bactrocera latifrons*, *Bactrocera zonata*, *Bactrocera tau*, and *Zeugodacus cucurbitae* were left in liquid baited traps for 7 days in outdoor conditions. We then dissected females and blindly determined their mating status. We identified which sperm storage organ (spermathecae or ventral receptacle (VR)) yielded the most accurate results, and for *B. dorsalis*, *B. latifrons*, *B. zonata*, and *B. tau* we evaluated the proportion of sperm stored in the VR.

Results: The type of bait used affected the correct assessment of mating status in *A. ludens* and *A. obliqua*, with traps baited with propylene glycol yielding the lowest correct results. Accuracy of mating status assessment was highest in traps baited with Ceratrap and Spinosad. Overall, it was easier to accurately detect an unmated than a mated female. For all species, accuracy was above 50% except for wild-mated *B. dorsalis* and *Z. cucurbitae*. We found the proportion of sperm stored in the VR varied from 10 to 80% depending on species.

Conclusions: Revision of the ventral receptacle is important. The use of aceto-orcein as dye did not improve the correct assessment of mating, while bait type only affected a few species. Alternative methodologies such as molecular techniques or machine learning could be implemented for more accurate determination of mating status, particularly for *B. dorsalis* and *Z. cucurbitae*.

Key words: Mating, *Bactrocera*, *Anastrepha*, *Ceratitis*, baits, sperm

Genetic-Ecological Integration for Adaptive Potential Assessment: Case Studies of Two Invasive *Bactrocera* species

Yujia Qin, Yuan Zhang, Zhihong Li

State Key Laboratory of Agricultural and Forestry Biosecurity, MARA Key Lab of Surveillance and Management for Plant Quarantine Pests, College of Plant Protection, China Agricultural University, Beijing 100193, China. E-mail: qinyujia@cau.edu.cn

Background: Most global warming is attributed to increased human activities, and this warming is likely to reshape how we perceive and manage nonnative invasive species. Species distribution models (SDMs) are widely used to assess invasion risks; however, they may underestimate threat levels because they do not account for drivers of local adaptation and evolutionary mechanisms that influence species' responses to environmental change.

Methods: To address this limitation, we developed an integrative and scalable framework that combines population genomics with climate niche modeling, supported by an RGB visualization approach, to improve the accuracy and resolution of invasion risk forecasts. We tested this framework on two invasive fruit fly species with contrasting distribution ranges: *Bactrocera tsuneonis* (restricted) and *Bactrocera dorsalis* (widespread).

Results: Using whole-genome resequencing data, we found that human-mediated dispersal has played a central role in their invasion history. Genotype–environment association (GEA) analyses identified candidate genes associated with climatic adaptation. Gradient forest models were then applied to quantify local, forward, and reverse genetic offsets, while SDMs were used to project current and future climatic suitability. Genomic offset analyses, together with SDM projections, were used to assess adaptive potential under climate change. Our results identify the most robust adaptation areas worldwide and reveal potential future migration directions.

Conclusions: These findings demonstrate the value of combining genomic and ecological data. By integrating genomic signals with climatic suitability, our framework captures adaptive capacity patterns that conventional SDMs alone may overlook, enabling more precise identification of high-risk regions and migration direction. This integrative approach provides a powerful tool for improving global risk assessments of biological invasions.

Key words: *Bactrocera tsuneonis*, *Bactrocera dorsalis*, Genotype–environment association, species distribution model, risk assessment

Session. Risk Assessment, Quarantine and Post-harvest

The Technical Panel on Phytosanitary Treatments (TPPT) and the Potential for New Harmonized Post-harvest Treatments targeting fruit fly species

Vanessa Simões Dias, Takashi Kawai, Peter L. Leach, Scott Myers, Meghan Noseworthy, Michael Ormsby, Daojian Yu, Guoping Zhan

Insect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Wagramer Strasse 5, 1400 Vienna, Austria. E-mail: V.Dias-de-castro@iaea.org

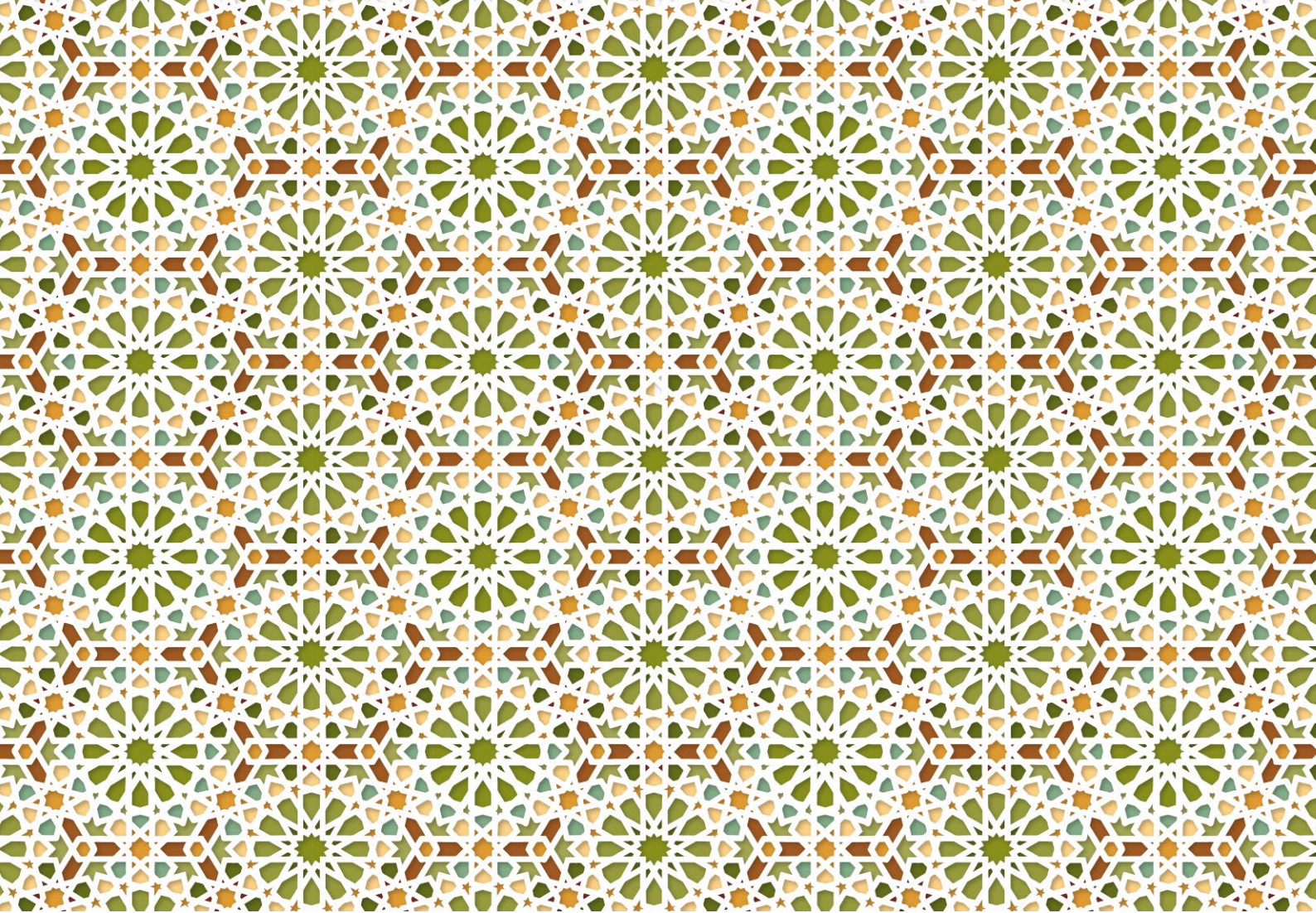
Background: The International Plant Protection Convention (IPPC) establishes expert working groups to address specialized phytosanitary topics and to develop International Standards on Phytosanitary Measures (ISPMs). One such group, the Technical Panel on Phytosanitary Treatments (TPPT), provides technical guidance to the IPPC Standards Committee (SC) on phytosanitary treatments, particularly those developed under ISPM 28. Although substantial advances have been achieved in phytosanitary irradiation treatments for fruit flies, opportunities remain to expand the range of approved post-harvest treatments.

Methods: Since its establishment in 2004, the TPPT has assessed phytosanitary treatment submissions from member countries in accordance with the requirements of ISPM 28 (Phytosanitary treatments for regulated pests). Submissions are reviewed following formal procedures and must be submitted through National or Regional Plant Protection Organizations.

Results: Between 2004 and the present, the TPPT evaluated more than 120 treatment submissions from 23 countries. Of these, 46 treatments were adopted as annexes to ISPM 28. Treatments targeting tephritid fruit flies accounted for 65% (30 treatments) of the adopted measures, reflecting both the economic significance of this pest group and the robustness of the supporting scientific data. A major milestone was the adoption of the first generic irradiation treatment (150 Gy) for all Tephritidae fruit flies. More recently, progress has been made toward removing restrictions on irradiation treatments for tephritid host commodities stored under modified atmospheres.

Conclusions: Given the global economic importance of fruit production and trade, additional submissions addressing temperature-based treatments for tephritids are encouraged. Expanding the portfolio of approved treatments targeting fruit flies under ISPM 28 will support safe international trade while strengthening plant health protection.

Key words: phytosanitation, harmonization, quarantine treatment, ISPM 28



ORAL COMMUNICATIONS

Sterile Insect Technique

The sterile insect technique: from conceptualization to global deployment

Kostas Bourtzis¹, Marc F. Schetelig^{2,3}, Walther Enkerlin¹, & Rui Pereira¹

¹Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Austria; ²Department of Insect Biotechnology in Plant Protection, Justus-Liebig-University Gießen, Institute for Insect Biotechnology, Winchesterstr. 2, 35394 Gießen, Germany; ³Liebig Centre for Agroecology and Climate Impact Research, International Atomic Energy Agency Collaborating Centre, Justus-Liebig-University Gießen, 35394 Gießen, Germany. R.Cardoso-Pereira@iaea.org

Background: Insect species represent an important component of the planet's ecosystem. Most of them are beneficial; however, a small number of them are major pests and disease vectors threatening horticulture, livestock and human health. Populations of insect pests and disease vectors were commonly treated with insecticides. The irrational use of insecticides has triggered the evolution of insecticide resistance. In addition, the insecticides have a significant negative impact on human health and the environment. Taken together, these facts have prompted the need for alternative, eco-friendly, safe, and sustainable methods for the population control of insect pests and disease vectors, such as the sterile insect technique (SIT).

Methods: The SIT consists of the mass-production, radiation-induced sterilization, packaging, handling, transportation, release and field monitoring of sterile insects of a target species over a defined area. The SIT should ideally be used as a component of area-wide integrated pest management (AW-IPM) projects under four different strategic approaches: suppression, eradication, containment, and prevention. The availability of genetic sexing strains (GSS) allows the release of sterile males-only rendering SIT applications more cost-effective.

Results: The first radiation-induced insect sterilization was against the cigarette beetle, *Lasioderma serricorne*, in 1916, while the first release of sterile insects was against the New World Screwworm, *Cochliomyia hominivorax*, in 1951, in Sanibel Island, Florida, USA. Since then, SIT has been applied against numerous plant pests, livestock pests, and human disease vectors in numerous countries on almost all continents. A historical overview of these projects will be presented, with examples from all strategic approaches, while lessons learned from failed projects will be discussed.

Conclusions: The SIT has been proven to be a very successful population control strategy against a variety of insect pests and disease vectors during the last seven decades. Despite its proven efficiency, challenges may arise during both the development and implementation phases. Recent technological developments can enhance its efficiency and cost-effectiveness. We should also be aware that SIT is not a stand-alone method and cannot be applied against every insect pest species.

Key words: area-wide integrated pest management, insect pests, disease vectors, genetic sexing strains.

Metabolic Insights into Male Quality for the Sterile Insect Technique

Andrea Moyano¹, Marcella Bonanomi², Tecla Aramini², Elena I. Cancio Martinez³, Gülizar Pillwax³, Anna Cleta Croce¹, George Tsiamis⁴, Daniela Gaglio², Kostas Bourtzis³ & Francesca Scolari¹

¹IGM-CNR, Via Abbiategrasso 207, 27100 Pavia, Italy; ²IBSBC-CNR, Via Fratelli Cervi 93, 20054, Segrate, Italy; ³Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, P.O. Box 100, 1400 Vienna, Austria; ⁴Laboratory of Systems Microbiology and Applied Genomics, Dept. of Sustainable Agriculture, University of Patras, 2 G. Seferi St., 30100, Agrinio, Greece. E-mail: francesca.scolari@cnr.it

Background: The Sterile Insect Technique (SIT) is a cornerstone of area-wide integrated pest management programmes targeting tephritid fruit flies. Its success relies on the biological quality and mating competitiveness of mass-reared sterile males. Current quality control (QC) protocols provide a solid framework for monitoring key traits, such as emergence, flight ability, and selected reproductive parameters. Complementary approaches capable of offering more direct and rapid insights into male physiological and reproductive status are increasingly relevant, particularly in the context of improved diets, irradiation protocols, and strain development.

Methods: We investigated the use of metabolomics as a complementary tool for assessing male quality in SIT programmes. A large-scale liquid chromatography–mass spectrometry (LC–MS) pipeline was established to characterize the metabolic profiles of the male reproductive organs and gut of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera zonata*. Over 6,000 samples were analyzed across species, strains, and treatments, including larval diet supplementation and irradiation. Standard quality parameters, including emergence and flight ability, were measured in parallel to integrate the biochemical and phenotypic data.

Results: Comparative analyses revealed species- and strain-specific metabolic signatures, along with reproducible metabolic shifts associated with laboratory colonization and irradiation. Preliminary analyses identified candidate metabolic pathways linked to reproductive and energetic status, supporting the feasibility of developing metabolomics-based biomarkers to complement standard SIT QC metrics.

Conclusions: Integrating metabolomic fingerprints with established QC parameters offers a promising complementary approach for evidence-based SIT optimization. Metabolomics-derived markers may assist in evaluating production changes, comparing strains, and refining the rearing protocols. While further validation is ongoing, this strategy has the potential to strengthen existing QC frameworks and support the consistent production of competitive sterile males in tephritid species. Funding for this study was provided by REACT (Horizon Europe, 101059523).

Key words: Metabolomics, tephritids, quality control, *Bactrocera dorsalis*, *B. zonata*

Novel female attractant for economically important fruit fly species – multi-species and multi-location evaluation

Nayem Hassan¹, Abu Imroz Ali¹, Selcuk Hazir²

¹ Russell IPM R&D Team, Russell IPM Ltd, 45 First Avenue, Deeside Industrial Park, Deeside, CH5 2NU, United Kingdom

² Adnan Menderes University, Department of Biology, Aydın, Turkey
E-mail: nayem@russellipm.com

Background:

Tephritid fruit flies are major pests of horticultural crops, causing yield losses of up to 70% without effective control. Increasing restrictions on insecticides and the limited effectiveness of male-targeted lures have increased interest in female-based attract-and-control strategies. Biodelear is a novel female attractant previously shown to be effective against several key fruit fly species.

Methods:

Field trials were conducted during 2023–2024 across multiple Agro-Ecological regions. Target species included *Bactrocera dorsalis*, *Zeugodacus cucurbitae* and *Bactrocera zonata*, with additional trials against *Ceratitis capitata* and *Carpomya incompleta* in Turkey, and confirmatory trials against *C. capitata* in Australia. McPhail traps baited with Biodelear were compared with hydrolysed protein lures and conventional control programs.

Results:

Across all species and locations, **Biodelear** consistently attracted higher numbers of female fruit flies compared with standard protein lures. In Turkish trials, populations of *B. zonata* and *C. incompleta* were reduced by **40–60%**, with corresponding **80-93% reductions in fruit infestation**. Similar trends were observed for *B. dorsalis*, *Z. cucurbitae*, *B. tryoni* and *C. capitata*. Performance was consistent across diverse climatic conditions.

Conclusions:

Multi-species and multi-location evaluations confirm **Biodelear** as an effective, low-toxicity and cost-efficient female attractant for major fruit fly pests, including *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, *Bactrocera zonata*, *Bactrocera tryoni*, *Carpomya incompleta* and *Ceratitis capitata*. Its ability to reduce female populations and fruit damage supports its integration into mass trapping, attract-and-kill and bait spray strategies, with strong potential for area-wide IPM programs.

Key words: Female attractant, fruit flies, *Bactrocera*, *Ceratitis*, IPM, mass trapping

Improve *Drosophila suzukii* Male Sorting Efficacy for the Sterile Insect Technique using AI in a real-case of only sterile male releases

Gerardo de la Vega. (1) Lihuen Soria-Mercier (2), Lorena Franco (3), Wilson Edwards (4), Federico Napolitano (3), Francisco Azzaro (4), Federico Triñanes (5), Santiago Masague (5), Analía Mattiacci (1), Damian Corzi (3), José Lipovetzky (3), Luciano Smith (6), Juan Corley (1,2).

(1) Instituto de Investigaciones Forestales y Agropecuarias de Bariloche. Instituto Nacional de Tecnología Agropecuaria – Consejo Nacional de Investigaciones Científicas y Técnicas (IFAB, INTA–CONICET). San Carlos de Bariloche, Río Negro, Argentina. (2) Centro Regional Universitario Bariloche, Universidad Nacional del Comahue CRUB (UNCOMA). San Carlos de Bariloche, Río Negro, Argentina. (3) Centro Atómico Bariloche (CAB). Comisión Nacional de Energía Atómica (CNEA). San Carlos de Bariloche, Río Negro, Argentina. (4) Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA). Centro Regional Patagonia Norte. San Carlos de Bariloche, Río Negro, Argentina. (5) Laboratorio de Ecología Química, Facultad de Química, Universidad de la República (UdelaR). Montevideo, Uruguay. (6) Facultad de Ingeniería Universidad de Buenos Aires. Mail: delavega.gerardo@inta.gob.ar

Background: Invasive insects can cause significant economic impacts to agriculture worldwide and impact human health. Traditional pest management methods that include chemical insecticides have raised increasing environmental and health concerns, prompting the need for sustainable alternatives. The Sterile Insect Technique (SIT) which consists of releasing sterile males of a target pest, to mate with wild females is held as a promising solution. However, the success of SIT relies on the release of sterile males. Efficient separation of sexes prior to sterilization and release is necessary.

Methods: This study presents SIT-ia, a software–hardware system that utilizes artificial intelligence (AI) and computer vision to automate the sex-sorting process. We showcase its use with the fruit fly pest *Drosophila suzukii* (matsumura). Also, we present the use in a real-case of an open field pilot trial. We conducted this management technique on a highly diverse berry farm in northern Patagonia (Argentina) on a small agroecological farm where no insecticide is allowed.

Results: The system was able to identify males from females with a 98.6% accuracy, sorting 1000 sterile flies in ~70 min, which is nearly half the time involved in manual sorting by experts (i.e., ~112 min). Also, the SIT-treated plot exhibited a flatter population growth curve compared to adjacent plots, which may indicate suppression in the treated site. While the fecundity of wild-caught females did not differ between sites, a trend towards reduced fertility was observed in the SIT plot, suggesting successful field mating between sterile males and wild females.

Conclusions: This simple device can easily be adopted in SIT production protocols, improving the feasibility and efficacy of improved pest management practices. While the results of sterilized males provide empirical evidence for the use SIT in an open-field context.

Key words: *Drosophila suzukii*, Spotted-wing *Drosophila*, AI sex-sorting, low-cost SIT

Evaluating the effectiveness of the sterile insect technique using a state-space model of trap catch dynamics

Atsushi Honma^{1,2,3}

¹Okinawa Prefectural Plant Protection Center (OPPPC), ²Ryukyu Sankei Co., Ltd, ³Faculty of Agriculture, University of the Ryukyus, 123 Maaji, Naha City, Okinawa, Japan. E-mail: kishabmi@pref.okinawa.co.jp

Background: In the sterile insect technique (SIT), which suppresses pest populations by releasing large numbers of sterilized males to disrupt mating among wild insects, control effectiveness is determined by two key factors: (1) the supply of sexually mature sterile males (S) relative to wild males (N), and (2) the mating competitiveness of sterile males relative to wild males (c). Accurate estimation of these parameters is therefore essential. Factor (1) can be partially inferred from the ratio of sterile to wild males captured in monitoring traps (S/N ratio). In contrast, factor (2) has been estimated only by extrapolating results from mating competition experiments conducted in cages. However, the mating competitiveness of sterile males is known to decline as the spatial scale of experimental cages increases, suggesting that cage-based estimates may be substantially overestimated. In this study, I aimed to develop a statistical model to estimate the density suppression effect of SIT by inferring sterile and wild insect densities and the mating competitiveness of sterile males from the temporal dynamics of trap catches obtained through routine monitoring surveys.

Methods: I constructed a state-space model based on the numbers of sterile males (S) and wild males (N) recovered from monitoring traps at regular intervals. In the state process, the estimated number of wild insects around each trap was assumed to be determined by recruitment proportional to the estimated population size in the previous generation and by mortality occurring at a constant rate since the previous trap collection. Reproductive suppression was incorporated by assuming that competition between sterile and wild males for mating with females reduces the population growth rate in proportion to $N/(N+cS)$. The model was fitted to data obtained from the ongoing eradication program targeting the pumpkin fruit fly *Zeugodacus tau* in Okinawa, Japan.

Results: Model fitting yielded an estimated median value of mating competitiveness (c) of approximately 0.3 for sterile males. As expected, this value was substantially lower than those typically inferred from conventional cage experiments. Based on this estimate, we evaluated the expected suppression of adult population growth as a function of the S/N ratio. The results indicated that population growth could be reduced by approximately half when the S/N ratio reached around 4, and to about one quarter when the ratio reached approximately 10.

Conclusions: The statistical model developed in this study enables, for the first time, estimation of the mating competitiveness of sterile males relative to wild males under field conditions. Furthermore, the model allows adaptive management of sterile insect release numbers based on quantitative estimates of control effectiveness.

Key words: *Zeugodacus tau*, Pumpkin fruit fly, State-space model

Establishment and Functioning of a Sterile Medfly Unit in Morocco

Siham BOUJDIG, Anass EL AMRANI

National Office of Food Safety, Avenue Hadj Ahmed Cherkaoui, Agdal, Rabat, Morocco, Email: siham.boujdig@onssa.gov.ma

Background: The Mediterranean fruit fly, *Ceratitis capitata*, remains one of the most destructive pests affecting citrus production in Morocco, causing significant economic losses and posing a major constraint on exports. As part of efforts to strengthen Morocco's national integrated pest management strategy, the Sterile Insect Technique (SIT) was adopted because of its proven effectiveness and environmental sustainability.

Methods: Introduced in Morocco in 2006, this technique was first implemented through a programme involving the release of sterile male pupae in a 4,600-hectare pilot area in the Souss-Massa region. In 2017, the programme was extended to the Berkane region over 1,200 hectares, bringing the total treated area to 5,800 hectares. In light of the encouraging results obtained in reducing pest populations, the development of a national production capacity became a priority.

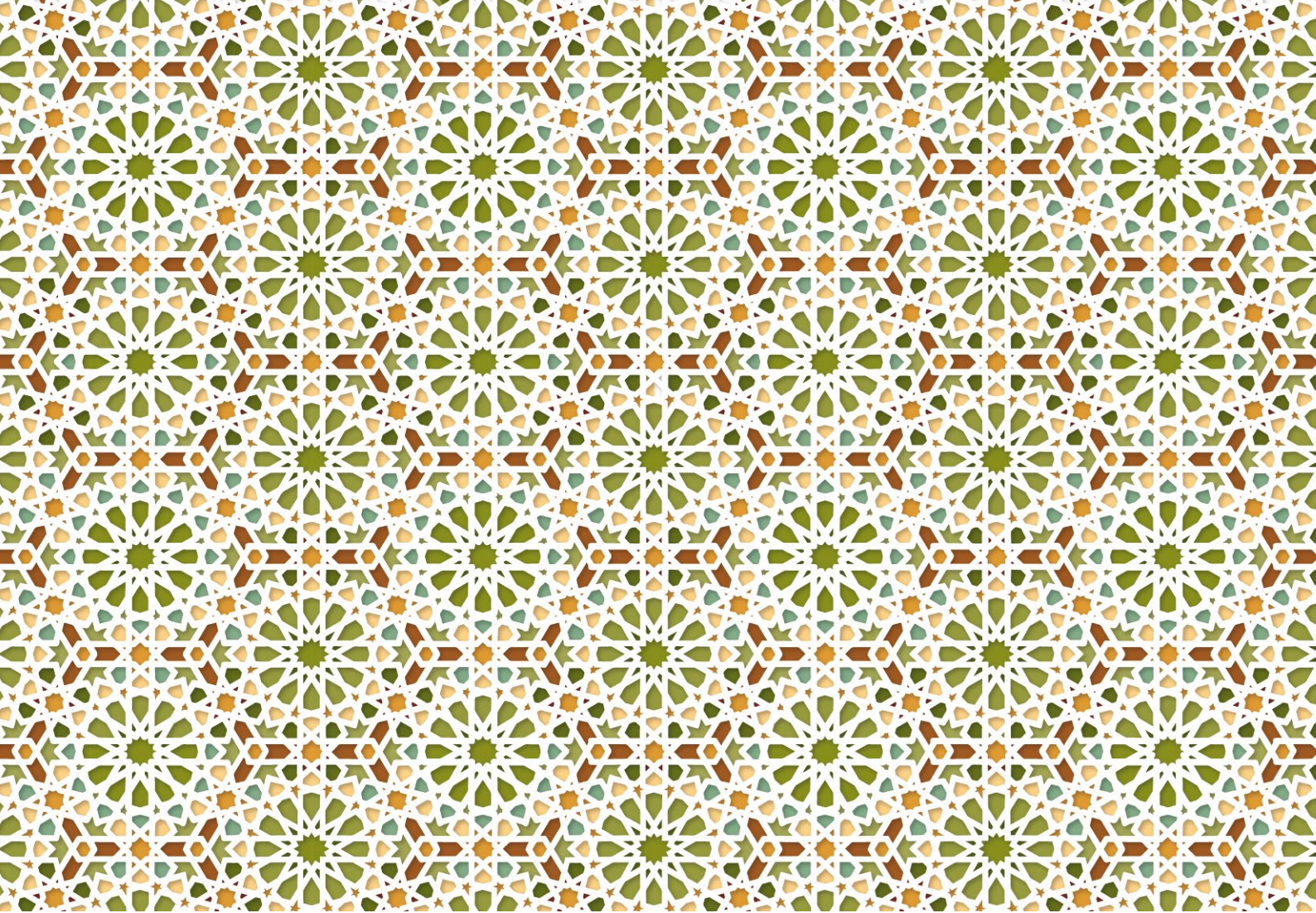
To this end, a facility dedicated to the mass rearing of sterile male Mediterranean fruit flies was established through the construction of the required infrastructure, the acquisition of rearing equipment, and the importation of a cobalt-60 irradiator for pupal irradiation. In parallel, specialised training was provided to strengthen the technical capacities of staff involved at the different stages of production.

Results: The unit is currently operational under the management of Morocco Citrus, the Moroccan interprofessional citrus organisation, with an estimated production capacity of 15 to 20 million sterile pupae per week. The support of public institutions, notably the National Office of Food Safety (ONSSA), the technical assistance of the International Atomic Energy Agency (IAEA), and the active involvement of the private sector were key factors in the successful implementation of this project.

Conclusions: In the long term, the objective is to increase production capacity to more than 350 million sterile pupae per week, in order to extend sterile male releases to all citrus-growing regions of Morocco within the framework of a sustainable and integrated pest management strategy. The establishment of this unit therefore represents a major step towards national self-reliance in the production of sterile insects and an important lever for strengthening the phytosanitary protection of Moroccan citrus in an environmentally sustainable manner.

Keywords: Mediterranean fruit fly, *Ceratitis capitata*, Sterile Insect Technique, mass rearing, irradiator, Morocco, integrated pest management.

Session 6. Sterile Insect Technique



ORAL COMMUNICATIONS

Natural Enemies and Biological Control

Do natural products & synthetics offer solution to fruit fly management? Academic and industry perspective

Junaid U Rehman^{1,2,3} & Ghulam Jilani³

1 National Center for Natural Products Research, The University of Mississippi, Mississippi 38677, USA

2 MURI Innovations (www.muriinnovations.com)

3 Insect Pest Management Program, National Agricultural Research Center, Islamabad, Pakistan

Email: jur@muriinnovations.com, jrehman@olemiss.edu

Background: Any natural product ‘NP’ repellent to pest & non-repellent to its natural enemies can facilitate biological control & NP compatible IPM models. In our studies, *Peganum harmala* L. ‘PH’ was strongly repellent to *Bactrocera zonata* & *B. oleae* while non-repellent to its larval-pupal parasitoid *Psytalia concolor*. PH is a common herb native to arid & semiarid regions of Central Asian deserts, but now widespread throughout North Africa, the southwestern United States, & northern Mexico & rich source of alkaloids, especially harmaline, harmine, harmalol, and harmol. The *B. zonata* is one of the most harmful species of Tephritidae causing 25-50% losses fruit yield in Asia & a serious pest of peach & mango in Pakistan. In Pakistan, the fruit fly complex may cause losses that range between 20-90 % in different areas of the country, amounting to approx. rupees seven billion. *Dirhinus giffardii* is an aggressive pupal parasitoid of *B. zonata* & if undisturbed by PH then can be an effective component of IPM Layered Strategies at 500 acres mango orchard trials against *B. zonata*.

Methods: NP toxicity to FF pupation was tested by treating soil, and against parasitoids by surface deposits on filter paper in choice and no choice test. Synthetic pesticide ‘R’ was evaluated in soil deposits only. IPM models were designed with IPM Layered Strategies and executed at 500 acre of mango orchard. MAT, BAT, Sanitation, and NP were used in combination.

Results: EPH (80-90%) and NSO (70-75%) didn’t affect parasitism in choice, no choice test with single and group of wasp bioassay settings. Delayed pupation in acetone extract of PH observed & inhibited adult emergence of *B. zonata* by 30% at lower doses of 250 & 500 ppm. Synthetic pesticide ‘R’ induced 100% mortality to pupating larvae. IPM: In our field studies at 500 acres mango orchard, mass release of wasp worked effectively along with EPH sprays .

Conclusions: NPs, synthetic pesticides and each of IPM component has a limitation, by combining workable strategies management of FF is possible. These IPM Layered Strategies are specific to location.

Key words: *Bactrocera zonata*, *Dirhinus giffardii* Queens, IPM Layered Strategies

Thermal requirements and phenology modelling of *Drosophila suzukii*: implications for pest risk under changing climates

Shepard Ndlela, Esther Awuori, Abdelmutalab G A Azrag, Farid Ibrahim Maholidy & Samira A. Mohamed

International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya

Background: *Drosophila suzukii* (spotted-wing drosophila) is an invasive pest responsible for substantial economic losses in fruit crops worldwide. Following its recent invasion into Africa, the species is rapidly spreading, raising concerns about its establishment and impact under current and future climate conditions. Understanding its temperature-dependent biology is critical for predicting population dynamics and invasion risk.

Methods: Temperature-dependent life table parameters, including developmental time, survival, fecundity, and adult longevity, were quantified for an African *D. suzukii* population collected in Kenya. Experiments were conducted under constant temperatures ranging from 10 to 30 °C, as well as under fluctuating temperature regimes. Linear and nonlinear temperature-response models were developed using ILCYM software to characterize thermal effects on life history traits. Phenology and population growth were simulated, and global spatial risk indices, Establishment Risk Index (ERI), Generation Index (GI), and Activity Index (AI), were projected under current and future climate scenarios (2061-2080).

Results: Developmental thresholds were estimated at 6.87 °C for the egg–larva stage and 9.13 °C for the pupal stage. Optimal development and reproduction occurred between 20 and 27 °C, while mortality increased at thermal extremes, with minimum mortality near 19.9 °C. The highest intrinsic rate of natural increase ($r_m = 0.276 \text{ day}^{-1}$) and finite rate of increase ($\lambda = 1.317$) were observed at 27 °C. Population growth declined sharply above 30 °C, indicating a narrow thermal window for population viability. Spatial projections revealed potential poleward and elevational shifts in suitable habitats, including expansion into northern Europe, southern Canada, and African highlands, alongside contraction in overheated lowland regions.

Conclusions: The study highlights the strong influence of temperature on *D. suzukii* population dynamics and identifies clear thermal limits that may constrain or shift its distribution under climate warming. Region-specific, temperature-driven phenology models are essential for forecasting invasion risk and informing climate-smart pest management strategies. Integrating empirical life table data with mechanistic modelling provides a robust framework for optimizing surveillance and control of *D. suzukii* in Africa and globally under changing climatic conditions.

Key words: Spotted-wing drosophila, ILCYM, invasive pest, temperature-dependent life table, population dynamics; berries

Optimisation of Laboratory Rearing of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) for Augmentative Biological Control of *Ceratitis capitata* (Diptera, Tephritidae).

Elaini Rachid, Yahia Rahal and Ahmed Mazih

Agronomic and Veterinary Hassan II Institute, Agadir, MOROCCO

ahmedmazih@gmail.com

Background: Current control tactics against Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), rely primarily on chemical and mass trapping approaches. The introduction of a new parasitoid, *Diachasmimorpha longicaudata*, has proven necessary to overcome the low effectiveness of the only parasitoid species of *Ceratitis capitata* recorded in Morocco, *Psytallya (Opus) concolor*.

Methods: The braconid *Diachasmimorpha longicaudata* (Ashmead) a larval-pupal endoparasitoid, was reared on wild strain of *Ceratitis capitata*, collected from infested fruits of *Argania spinosa* (Sapotaceae). Rearing was conducted under restricted conditions, in the perspective of its probable release in the field. Medfly and parasitoid rearing were kept in a temperature-controlled insectary room ($25 \pm 1^\circ\text{C}$, $65 \pm 10\%$ RH; 12L:12D).

Reproductive and demographic parameters have been studied in order to optimize and improve rearing outcomes including 1) density and quality of host larvae, 2) host exposure time to the parasitoid, 3) sex ratio of parasitoid and ratio of host/parasitoid.

Results: Our findings indicated that the highest parasitism rate and parasitoid offspring production were obtained with ratios of 1:1 and 2:1 (larvae/female), along with the parasitoid lowest pupal mortality level, and the highest flight ability, at 24h exposure duration. A diet of pure honey and water for adult parasitoids gives a high longevity of almost 70 days. No significant differences were observed among the remaining parameters across experimental treatments.

Conclusions: *D. longicaudata* is considered among the most successful parasitoid species currently used in biological control programs against the Mediterranean fruit fly. However, its success as a biological control agent depends on the ability to mass-rear and deliver large numbers of high-quality individuals for field release. Our results provide data and optimized rearing protocol that can enhance the efficiency and reliability of *D. longicaudata* mass-production for biological control applications.

Key words: Biological control; *Ceratitis capitata*; *Diachasmimorpha longicaudata*; parasitoid; Morocco.

Session: Natural Enemies and Biological Control

Identification of fruit fly pupae parasitized by *Doryctobracon areolatus* (Hymenoptera: Braconidae) using radiographic images

Lorena Quintero-Rodríguez¹, Sandro Nornberg², Rafael Gonçalves², Ana J. Prestes³, Maria L. Z. Costa³, Clissia B. Mastrangelo³, Dori E. Nava¹ & **Thiago Mastrangelo**³

¹EMBRAPA Clima Temperado, km 78 Monte Bonito, Pelotas, 96010-971, RS, Brazil.

²PARTAMON Parque Tecnológico Room 4i, Av. Domingo de Almeida 1785, Pelotas, 96085-470, RS, Brazil.

³Centre for Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP), Av. Centenario 303, Piracicaba, 13416-000, SP, Brazil. E-mail: piaui@cena.usp.br

Background: In the last 10 years, research efforts have been made in Brazil to stimulate the biological control of fruit flies with native species, such as *Doryctobracon areolatus* (Hymenoptera: Braconidae). Recent tests at CENA/USP to optimize the mass production of this parasitoid in the laboratory have shown that this wasp can also develop normally in 2nd instar larvae originating from eggs irradiated with 20-24 Gy of X-rays, which prevents the emergence of adult flies from non-parasitized pupae. Despite this advantage brought by this radiation technique, it is only possible to determine the percentage of parasitism after the emergence of adults from sampled pupae. One way to get around this problem would be to combine digital radiography and deep learning methods for the rapid classification of images of parasitized pupae. Therefore, the purpose of this work was to distinguish parasitized from waste pupae through X-ray images and Convolutional Neural Networks (CNNs).

Methods: Larvae of *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae) and *D. areolatus* adults used for the experiments were obtained from the CENA's colonies. Eggs of these two species of fruit flies were irradiated with 24 and 20 Gy of X-rays, respectively, after 48 h of aeration in water at 24°C, and 4 mL aliquots of eggs were seeded in trays with 200 mL of CENA's standard larval diet. After 5 days of development, the 2nd instar larvae were separated and exposed to the parasitoid females (at a 10:1 ratio). After 6 h of parasitism, the larvae were returned to the diet to complete their development. Third instar larvae were placed in vermiculite to pupate, and pupae of different ages were taken for X-ray imaging.

Results: Radiographic images of the parasitized pupae of *C. capitata* and *A. fraterculus* with 8 and 11 days before emergence, respectively, allowed the *in vivo* identification of immature stages of the parasitoid (such as pre-pupae and pupae), preserving the viability of the wasp. Positive correlations were found between the number of emerged parasitoids and the ones identified in the X-ray images. Of the different CNN models pre-trained on the ImageNet dataset, the Xception and MobileNetV2 models were able to distinguish the parasitized from waste pupae with more than 97% accuracy.

Conclusions: Our results demonstrated that the combination of digital radiography and CNNs is a promising non-invasive technology to accurately classify pupae parasitized by *D. areolatus* in mass-rearing facilities, predicting the parasitism rate in advance without negatively affecting the viability of parasitoids.

Key words: Quality control, Radiography, Deep learning, Biological Control

Optimization of the rearing and release of *Psytalia lounsburyi* (Hymenoptera - Braconidae), a larval parasitoid of *Ceratitis capitata* infesting Citrus in Souss-Massa region

Zahidi Abdelaziz^{1,2*}, Aminzou Said², Leghlid Amine^{3,4}, Salhi Toufik³, Amazouz Said¹, El Khrouchni Toufik¹, Barakat Ilham⁵, Chtaina Nouredine⁵

¹ Laboratory of Biotechnology and Valorization of Natural Resources; Faculty of Science, Ibn Zohr University, Agadir, Morocco;

² Laboratory of Applied Entomology, Hassan II Agronomic and Veterinary Institute (IAV), Agadir Horticultural Complex (CHA);

³ Association of Producers of the Nadorcott Variety, Morocco (APMN).

⁴ Sterile Male Production Center, Agadir, Morocco

⁵ Phytopathology Laboratory, Hassan II Agronomic and Veterinary Institute, Rabat, Morocco.

* a.zahidi@cha-agadir.ma

Background: The Mediterranean fruit fly, *Ceratitis capitata*, is a major pest of *Citrus*, causing fruit damage through egg-laying and larval feeding that leads to rot and early fruit drop. In regions with Mediterranean-like climates, such as Souss-Massa, outbreaks can be severe. To reduce infestation and keep populations below economic thresholds, several phytosanitary measures have been implemented within an integrated pest management programme, including the use of parasitoids of the genus *Psytalia*.

Methods: The parasitoid's activity was monitored at different sites in the Argan forest while laboratory rearing conditions were controlled, with the aim of optimizing augmentative releases according to peaks in host population density. Rearing and exposure conditions were maintained at a temperature of 22–26 °C, with a relative humidity ranging from 60 to 75%, and a photoperiod of 12–14 h of light followed by 10–12 h of darkness. Host larvae were introduced into rearing boxes, after which adult female *Psytalia* aged 3–5 days were added. The exposure period lasted between 24 and 48 hours, using a recommended ratio of one parasitoid female per 10–20 host larvae.

Results: The highest parasitism rate was recorded at Ihchach (21.5%), followed by Amskroud and Admin forest (more than 5%). Higher rates occurred near agricultural areas. Natural mortality was also observed, likely due to temperature, box conditions, or parasitism. The exposure period lasted 24–48 hours with a ratio of one parasitoid female to 10–20 host larvae. Emerging adults were kept in ventilated cages and fed diluted honey. Key biological parameters—cycle duration, parasitism rate, emergence rate, and sex ratio—still need to be evaluated to assess the rearing system's performance.

Conclusions: Parasitoid rearing represents a promising and sustainable approach to biological control of *C. capitata*. The predominance of females and the synchronisation of parasitoid releases with peaks in host population density are essential factors in maximising the effectiveness of control.

Key words: *Psytalia lounsburyi* – Rearing - Release – Fruit fly – *Citrus* – Population density – Biological control

Entomopathogenic fungi against *Drosophila suzukii* and associated drosophilids: efficacy, selectivity and horizontal transmission of *Cordyceps javanica*

Ricardo Alberto Toledo-Hernández, Rodrigo Lasa, Pablo Montoya, Pablo Liedo, Daniel Sánchez, Mónica Pulido, Arturo Sánchez, Douglas Rodríguez & Jorge Toledo, PLANASA. Fuente el Olmo de Fuentidueñas, 40359 Segovia, Castilla y León, España. E-mail: rtoledo@planasa.com

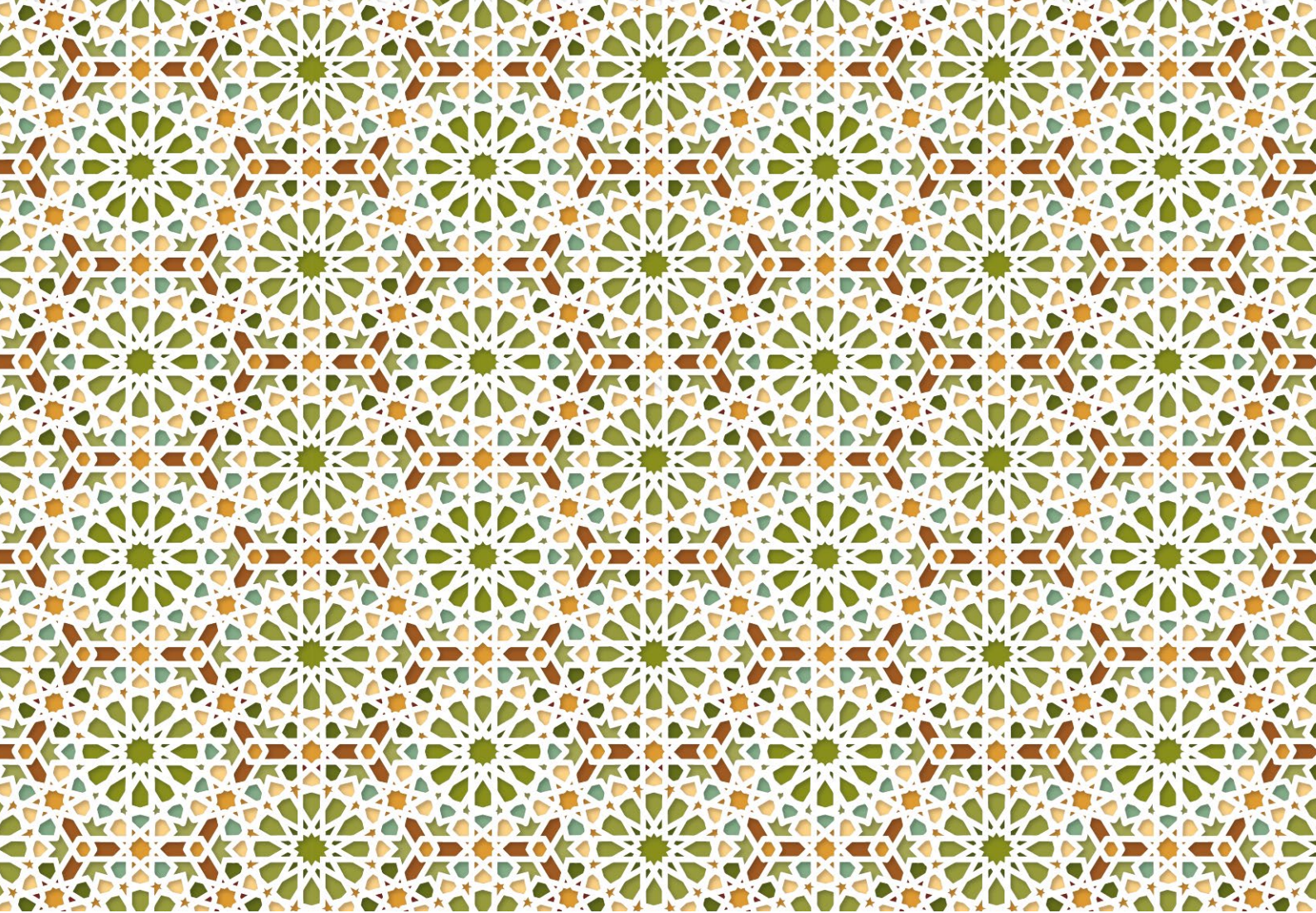
Background: *Drosophila suzukii* is an invasive pest of soft-skinned fruit crops in the Americas and Europe whose management relies largely on repeated insecticide applications, raising concerns about residues, resistance, and non-target effects in production systems where the pest co-occurs with other drosophilids, such as *Zaprionus indianus* and *Drosophila melanogaster*, and where pollinators like *Apis mellifera* are routinely introduced, creating a clear need for control tools that are effective against multiple drosophilids while remaining selective toward beneficial insects. In this context, entomopathogenic fungi have emerged as promising biological control agents, inducing lethal and sublethal effects that may contribute to population suppression and be influenced by horizontal transmission during mating or social interactions. Although horizontal transmission has been exploited in other dipteran pests, the efficiency, persistence and demographic relevance of *Cordyceps javanica* transmission in *D. suzukii* remain unexplored.

Methods: Fifteen commercial dry powder formulations of entomopathogenic fungi were evaluated against adult *D. suzukii* under laboratory conditions by exposing flies to dry conidia and assessing lethal responses. Based on virulence, selected formulations were further tested against *D. melanogaster*, *Z. indianus*, and the pollinator *A. mellifera* to assess efficacy and selectivity. Subsequently, *Cordyceps javanica* was selected as a representative formulation for detailed laboratory and semi-field experiments to quantify conidial acquisition, horizontal transmission during mating, and associated lethal and sublethal effects on adult survival and reproduction.

Results: Fungal formulations differed in adult mortality, with several products showing high efficacy against *D. suzukii* and associated drosophilids while exhibiting minimal impact on *A. mellifera*. Among these, *C. javanica* was selected for in-depth analysis. Self-inoculated adults exposed to *C. javanica* acquired $1.5\text{--}1.8 \times 10^8$ conidia and showed 87-93% mortality; during the first mating event, $1.2\text{--}6.0 \times 10^6$ conidia were transferred, resulting in 57-75% mortality in recipient flies. Transmission efficiency and fungal-induced mortality declined in subsequent matings. Infection reduced female fecundity by 27-58% without affecting fertility or mating behavior and semi-field mortality remained low, largely limited to females mating directly with inoculated males.

Conclusions: These results show that entomopathogenic fungi can provide effective and selective control of *D. suzukii* and associated drosophilids, supporting their integration into biologically based management strategies. Although *C. javanica* can be transmitted through mating, its epidemiological impact is limited because infectivity declines rapidly, secondary transmission is minimal and reductions in female fecundity do not prevent continued oviposition. Together, the findings highlight both the potential and the limitations of entomopathogenic fungi in berry crops.

Key words: *Drosophila suzukii*, Entomopathogenic fungi, Horizontal transmission, Sublethal effects



ORAL COMMUNICATIONS

Other Control Methods

Operational Integration of Unmanned Aerial Vehicles (UAV's) for Mediterranean Fruit Fly (*Ceratitis capitata* Wied) Control in Guatemala's Moscamed Program

David Dominguez¹, Pedro Rendon², Corinna Bazelet³, Alene Seiler⁴, Jorge Lopez⁵, Yeudiel Gomez⁶

Moscamed Program, 8 C 14-22 z13, Guatemala. E-mail: David.dominguez@medfly.org.gt

Background: Traditional methods for the application of food bait for the control of the Mediterranean fruit fly in Guatemala are primarily based on ground spraying using backpack equipment. This approach requires a large workforce, coordination of access with farm owners/producers, and presents operational limitations associated with topography, particularly in coffee-growing areas. By comparison, aerial spraying using fixed-wing or rotary aircraft involves high logistical costs as well as significant social and environmental considerations. More efficient, safe, and environmentally friendly solutions are needed.

Methods: Food bait applications using unmanned aerial vehicles (UAVs) were calibrated to spray a 30% bait dilution at a rate of one gallon per hectare. During these applications, a number exceeding 95 droplets per square meter, with diameters ranging from 3 to 6 mm, was achieved. To compare the performance and control parameters of this new application tool to conventional methods, a cost comparison was conducted.

Results: Implementation of UAVs for Mediterranean fruit fly control required adaptation of equipment to accommodate the physical characteristics of the food bait, including viscosity, application volume, droplet distribution and size, under complex operational conditions including rugged terrain and dense tree canopy cover. The calibrated application parameters reported here produced high control efficacy and showed an economic analysis with a benefit-cost ratio of >1.4:1, demonstrating an advantage of over conventional ground-based and aerial application methods. Additionally, this technology reduced social and environmental impacts by avoiding the use of airplane engines, minimizing the entry of personnel vehicles and frequent entry into production units, combined with limiting spray drift through precise control of flight height and speed.

Conclusions: The use of UAVs has emerged as a precision agriculture tool with low social and environmental impact. Bait application using UAVs demonstrated superior control efficacy, reinforced by improved coverage and effective droplet density. Furthermore, this technology reduces labor requirements, optimizes application time, and minimizes environmental impact, consolidating UAVs as an efficient and viable alternative within integrated Mediterranean fruit fly management programs.

Keywords: Unmanned aerial vehicles; *Ceratitis capitata* Wied; food bait; precision agriculture; integrated pest management.

Field dispersal and recapture patterns of the Mediterranean fruit fly: Roles of thermal acclimation and environment

Eleftheria-Maria D. Bali, Eleni Verykouki and **Nikos T. Papadopoulos**

Laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, School of Agricultural Sciences, University of Thessaly, 38446 N. Ionia (Volos) Magnesia. E-mail: nikopap@uth.gr

Background: Dispersal is a key ecological and evolutionary process influencing species range expansion, gene flow, and adaptation to changing environments. In insects, dispersal is shaped by biotic and abiotic factors, with temperature strongly affecting physiology, flight performance and habitat suitability. Thermal history can further modify dispersal through phenotypic plasticity; however, the effects of thermal acclimation on insect dispersal under field conditions remain poorly understood. This study addresses this gap using the Mediterranean fruit fly (*Ceratitidis capitata*), a globally invasive pest expanding into cooler regions, to examine how thermal acclimation and environmental factors interact to influence field dispersal.

Methods: Release–recapture experiments were conducted using laboratory-reared progeny (within? three generations) of a wild population from Volos, Greece. Trials took place in two contrasting habitats: a mixed-fruit orchard with host plants and an olive orchard without hosts. Trapping grids centered on release points were established using McPhail and Jackson traps at increasing distances. Two days before adult emergence, pupae were marked with different colors of a nontoxic fluorescent powder depending on the acclimation treatment they were slated to experience. The emerged adults were kept for five days at 25°C. Then, they were subjected to one of the three thermal acclimation treatments (20, 25 and 30°C) for 5 days. At the age of ten days, 100 individuals of each sex and treatment were released at the central point of each trapping grid. Four releases were conducted across autumn–winter and spring–summer periods. Recaptured adults were identified under ultraviolet light.

Results: Overall recapture rate was 14.8%, with males recaptured more frequently than females. Recapture rates increased with higher ambient temperatures and most flies recovered near release points and within ten days post-release. The presence of host plants was associated with higher recapture rates compared to host-free sites. Thermal acclimation alone did not affect recapture probability; however, flies acclimated at 30°C tended to be recaptured more frequently under higher field temperatures.

Conclusions: These findings improve understanding of temperature-driven dispersal under field conditions and have important implications for predicting invasion dynamics and developing sustainable pest management strategies in the context of climate change.

Key words: *Ceratitidis capitata*, mark-release-recapture, thermal history, environmental conditions

Citrus fruits produce direct defense responses against oviposition by *Bactrocera minax* (Diptera: Tephritidae)

Bingbing Wei, Guijian Zhang & Changying Niu

College of Plant Science & Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China. E-mail: niuchangying88@163.com

Background: Plants can perceive and initiate defense responses upon oviposition by herbivorous insects. However, how fruits—a key plant reproductive organ—respond to insect eggs remains poorly understood. Here, we investigate the molecular mechanisms underlying fruit defense elicited by egg deposition of the Chinese citrus fly, *Bactrocera minax*.

Methods: In this study, we performed transcriptome profiling to compare gene expression in *Citrus sinensis* fruits following oviposition by female flies. Concurrently, we quantified the accumulation of lignin, phytohormones, and secondary metabolites at the oviposition site using a combination of metabolomic, hormonal, and histochemical analyses. Furthermore, we assessed the impact of these induced substances on the survival and development of the eggs and subsequent larvae.

Results: We found that oviposition by the specialist insect *B. minax* in navel oranges activated the lignin synthesis pathway and cell division, causing mechanical pressure that crushed the eggs. Transcriptome and metabolome analyses revealed an enrichment of oviposition-induced genes and metabolites within the lignin synthesis pathway, which was confirmed by histochemical staining. Furthermore, hydrogen peroxide (H₂O₂) accumulation was observed at the oviposition sites. Plant defense-related hormones jasmonic acid (JA) and salicylic acid (SA) exhibited rapid induction after oviposition, while indole-3-acetic acid (IAA) activation occurred in the later stages of oviposition. Additionally, secondary metabolites induced by prior egg deposition were found to influence larval performance.

Conclusions: Together, our studies reveal that host fruits have evolved specific defense responses to insect eggs at the molecular level, which provides a critical basis for future strategies aimed at developing insect-resistant citrus varieties.

Key words: *Citrus sinensis*, plant defense, lignin biosynthesis, tephritid fruit fly, *Bactrocera minax*, oviposition

A Technically and Economically Feasible Approach to the Aerial and Ground Release of Sterile Insects for New Control Projects

Roberto Angulo-Kladt*, Marcos Astegiano*, Hector Santamarina**

*Air Evolution México. **Aerotec Argentina.

Background: In virtually all large-area integrated pest management projects, the sterile insect technique, as well as biological control, has proven to be one of the most efficient methods due to its safety and environmental friendliness. Both methods require the large-scale production of sterile adult and male individuals of the target pest. This implies efficient facilities under controlled conditions, which have gradually been standardized according to manuals developed primarily by the IAEA and some other countries with large-scale production, such as the USA, Mexico, Guatemala, Brazil, Spain and Argentina. Historically, these processes have required a large workforce due to the stages of mass rearing and, especially, quality control to obtain a vigorous adult that is competitive with the wild population.

Methods: All products developed in biofactories require, to achieve their objective, to be released in optimal conditions and without losing any attributes. They must be supported with optimal nutrition, aromatherapy, and hormones (if necessary) to avoid stress, and must be handled and transported to the release area in absolute safety and comfort. For this purpose, equipment has been developed that, until now, had very large capacities, able of transporting 5 to 10 million *Ceratitis capitata*, and released by air.

As the cost of aerial release increased significantly, smaller projects (less than 4000 ha) stopped being economically feasible. Today, new options have been created for this purpose such as ground-based machines and unmanned aerial vehicles, which are faster than ground-based release. The important thing now is the decision-making; that is, determining which of these release methods is most adaptable and efficient for small projects or pilot projects, scaling up to larger control areas in an economically feasible way. The following work aims to identify the evaluation metric for each project.

Conclusions: There are several successful and sustainable AWM programs against pests like fruit flies, mosquitoes and lepidoptera set up in different countries around the world like Chile, Galapagos, China and Mexico. It should be possible to set up a national fruit fly AWM program that could be used to establish management systems in new projects.

Key words: aerial release, chilled adult method, ground release, UAS release method

Aromatherapy effects in pre-release SIT in *Ceratitis capitata*: comparative transcriptomics of wild and Vienna 8 males

Brenda Torres-Huerta^{1,2}, Obdulia L. Segura-León¹, José S. Meza², Juan Cibrian-Tovar¹, Lauro Soto-Rojas¹, Yalbi I. Hernández-Martínez³

¹Fitosanidad, Colegio de Postgraduados Campus Montecillo, Texcoco de Mora, State of Mexico, 56264, Mexico, ²Departamento de Genética, Programa Operativo de Moscas, SADER-SENASICA/IICA, Metapa de Dominguez, Chiapas, 30860, Mexico, ³INER, Tlalpan, CDMX, 14080, México

Background: Aromatherapy with plant-derived essential oils is used as a pre-release treatment in the Sterile Insect Technique (SIT) to enhance male mating performance in *Ceratitis capitata*. Protocols differ among programmes; MOSCAMED Mexico applies Valencia orange peel oil (ORA), whereas MOSCAMED Guatemala uses ginger root oil (GRO). Despite extensive behavioural evidence, its physiological and molecular basis remains unresolved. Two main hypotheses have been proposed: a perfume-like effect that increases female attraction, and activation of chemosensory/endocrine pathways that modulates sexual signalling. This study compares head transcriptomic responses to ORA and GRO in wild F₁ males versus the mass-reared, irradiated Vienna 8 GSS, integrating OBP expression and sequence variation.

Methods: We generated 18 paired-end RNA-seq libraries from heads of sexually mature WT and Vienna 8 (V8) males exposed to GRO, ORA, or a non-aromatherapy control. Differential expression was assessed within genotype (DESeq2), and upregulated genes were analysed by GO enrichment with semantic-similarity reduction. Selected patterns were validated by semi-quantitative RT-PCR. OBP-focused variant calling/annotation was integrated with expression data to link transcriptional responses with chemosensory variation.

Results: Sequencing yielded high-quality reads (Q30 ~89–93%), with mapping to the *C. capitata* EGII genome of >90% in WT and ~50% in V8. PCA separated samples primarily by genotype (PC1 = 42%) and secondarily by treatment (PC2 = 13%). WT showed the strongest response (GRO highest) with substantial overlap with ORA, whereas V8 showed fewer upregulated genes, a preserved GRO response, and limited overlap between aromas. WT profiles were dominated by terpenoid/isoprenoid metabolism and xenobiotic detoxification with olfactory enrichment, while V8 lacked chemosensory enrichment; ORA showed a biotransformation/endocrine–metabolic signature, whereas GRO was characterized by neurostructural and kinase-modulatory functions (including PKC/Ser–Thr inhibitory activities). OBP analyses supported genotype divergence and detected OBP DEGs only in WT (OBP99c-like cluster and OBP19d), with none in V8.

Conclusions: Aromatherapy effects were genotype and oil dependent. V8 showed no chemosensory enrichment, whereas WT exhibited olfactory enrichment and OBP differential expression. In Vienna 8, ORA elicited a limited response, whereas GRO added a neurostructural signature, supporting GRO aromatherapy as a more robust pre-release option for SIT programmes.

Key words: genetic sexing strain, transcriptome, genetic variants, OBPs

SEMIOCHEMICAL-BASED TECHNOLOGIES FOR NO-GAP MANAGEMENT OF FRUIT FLY PESTS

Agenor Mafra-Neto¹, T. Dekker², C. Bernardi¹, V. Soria¹, R Machota Junior¹, L.C. Bortoli¹, J. Saroli², M.L. Martin¹, M. Tonelli¹, M. Lasiter¹, R. Pederson¹, K.L. Spencer²

¹ ISCA, Inc., 1230 W. Spring St., Riverside, CA, USA, 92507, ² ArcGen Pro Technologies LLC, MRB Building, 900 University Ave. Riverside, CA, USA, 92521

E-mail: amafra@isca.com

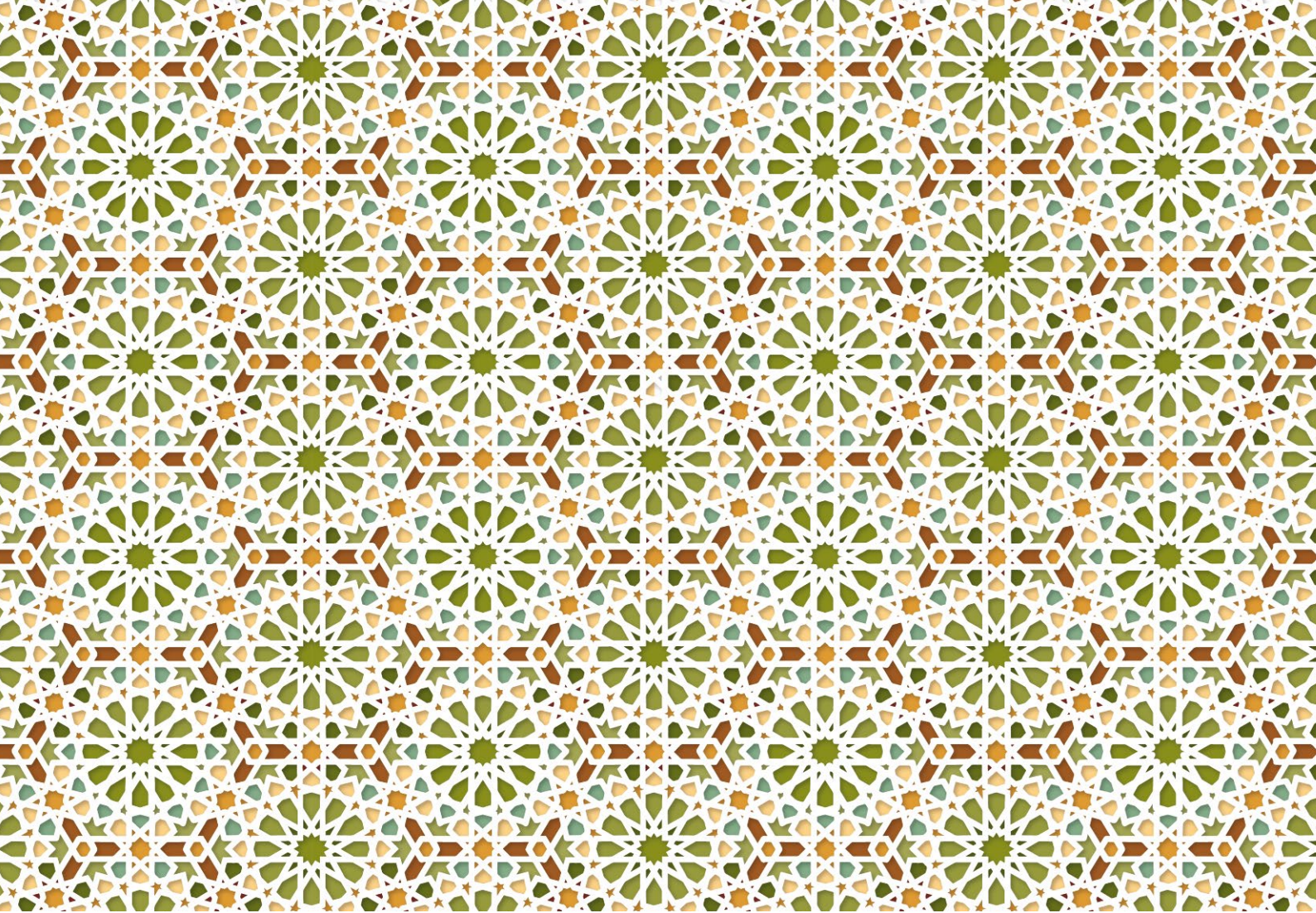
Background: True fruit flies (Diptera: Tephritidae) are among the most impactful agricultural pests globally, with approximately 350 species considered economically significant. Different species of these pests coexist, and together cause billions of dollars in annual losses. For instance, the Mediterranean fruit fly (*Ceratitis capitata*) alone is estimated to cause \$2 billion in global losses per year. In regions like West Africa, direct yield losses range from 30% to 95%, severely hindering export-oriented growth. Conventional control tends to rely heavily on conventional synthetic insecticide cover sprays, but these face challenges including non-target toxicity, environmental hazards, and the evolution of resistance. ISCA's "no-gap" areawide management strategy addresses these limitations through a program of long-lasting, flowable formulations. This approach establishes a baseline using Male Annihilation Technique (MAT) formulations that persist for up to 12 weeks, complemented by the application of Anamed, a general food attractant bait, every 3 weeks.

Methods: A suite of crop protection technologies using ISCA's proprietary SPLAT (Specialized Pheromone and Lure Application Technology) controlled-release matrix, a food-grade, thixotropic wax emulsion enables targeted semiochemical release periods and degrees of rain fastness. Formulations include Anamed WB SP01 (Anamed), a palatable new blend of protein-based semiochemical attractant bait containing spinosad designed for superior weather resistance compared to industry standards like GF-120. MAT formulations include SPLAT MAT ME (e.g., *Bactrocera dorsalis*, *B. zonata*, *B. carambolae*), HOOK CL (e.g., *B. tryoni* and *B. cucurbitae*), and HOOK TML (e.g., *Ceratitis* spp.). Furthermore, we utilized gas chromatography coupled to palpal detection (GC-EPD) and antennal detection (GC-EAD) to identify selective, non-toxic attractants for female flies. These formulations were tested in the US, Brazil, Peru, Italy, Spain, and South Africa.

Results: Field trials in South Africa revealed that Anamed achieved equivalent or superior suppression of fruit flies (*C. capitata*, *B. invadens*) and crop damage compared to standards, even when applied at half the frequency. In citrus, Anamed was 60% to 80% more effective at suppressing infestation than GF-120. In Brazilian apple orchards, Anamed reduced trap captures by up to 74%. HOOK MAT formulations maintained efficacy for up to 12 weeks, outperforming traditional naled-based liquid baits. Optimization studies in California and Florida validated the "MAT-ME saturation hypothesis," demonstrating that reducing point source density to 110/km² significantly increased recapture rates ($p=0.020$). Additionally, combined palpal and antennal lures attracted significantly higher numbers of female flies with the lowest rate of non-target bycatch.

Conclusions: Advanced semiochemical technologies like Anamed and SPLAT-MAT offer effective, reduced-risk alternatives for "no-gap" area-wide fruit fly management. By extending field longevity and optimizing application densities, the complementary effect of these technologies applied in tandem results in effective no-gap control, reducing operational complexity and costs. These innovations are critical for global biosecurity, particularly in resource-limited regions where stabilizing horticultural production is vital for food security and poverty reduction.

Key words: Tephritidae, area-wide management, attract-and-kill, semiochemicals, SPLAT, Anamed, Hook, *Ceratitis*, *Bactrocera*, *Anastrepha*



ORAL COMMUNICATIONS

Area-wide Integrated Pest Management and Action Programs

Advancing Area-Wide IPM for Mediterranean Fruit Fly: Lessons in Guatemala

Luis Avila¹, Vanessa Dellis², Jorge Lopez³, Roberto Gomez⁴

Moscamed Program, 8 C 14-22 z13, Guatemala. E-mail: luis.avila@medfly.org.gt

Background: The Mediterranean fruit fly (*Ceratitis capitata* Wied) – Medfly – was first detected in the Americas in Brazil in 1901 and later in Costa Rica in 1955, subsequently spreading throughout the region, reaching Guatemala in 1975 and Mexico in 1977. Due to its broad host range and the severe damage, it causes to fresh fruits and vegetables, Medfly has become one of the most economically and environmentally significant pests worldwide. Beyond regional impacts, its presence poses persistent challenges to horticultural production and international trade, triggering stringent quarantine measures and threatening food security.

The Moscamed Program, established through bilateral and now trilateral agreements among the United States, Guatemala, and Mexico, applies an area-wide integrated pest management (IPM) approach to control Medfly infestations. The program is anchored in the Sterile Insect Technique (SIT), supported by the largest mass-rearing facility for sterile Medfly, an emergence and release center, and field operation offices for surveillance and ground operations. While the program has incorporated advanced technologies to enhance efficiency, factors such as climate variability, cultural practices, and host plant distribution continue to pose challenges for detection and eradication efforts.

Methods: In Guatemala, Medfly populations exhibit a seasonal cycle, with low population levels during the first and fourth quarters, rapid population growth in the second quarter, and dispersal during the third quarter. This pattern enables strategic IPM planning, ensuring control activities occur when most effective. SIT remains the primary tactic, complemented by a strong detection network, cultural measures, chemical control, and internal quarantine control to achieve suppression and eradication.

Results: Despite record-high populations in northern Guatemala and southern Mexico in 2019–2020 caused by adverse weather and operational constraints, subsequent years saw a decline, although challenges resurfaced in 2024. In 2025, the implementation of all IPM components, guided by strategies jointly developed with partner countries, achieved a 94% population reduction. These efforts significantly improved phytosanitary conditions along the Mexico-Guatemala border and confined outbreaks to designated suppression zones, protecting the internationally recognized free area of Peten, Guatemala (29,500 km²), where only one transitory entry was recorded in 2025.

Conclusions: Through the timely deployment of activities and maintaining the Sterile Insect Technique (SIT) as the cornerstone of its area-wide strategy, the Moscamed Program has successfully prevented northward spread of Medfly into Mexico and the United States. The technical strategy implemented by the Moscamed Program demonstrates that sustained application of the (SIT), integrated within an area-wide IPM framework, can achieve substantial suppression of Medfly populations even under challenging ecological and operational conditions.

Keywords: *Ceratitis capitata* Wied, Mediterranean fruit fly, area-wide management, Sterile Insect Technique.

Modelling-Informed Decision-Making in Guatemala's Medfly Program

Estuardo Lira, Alene Seiler-Martinez, Gwen Keller[†]

U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS),
Action Programs, Medfly Program (Guatemala, Mexico, Belize), U.S. Embassy, Blvd. Austriaco
11-51, Guatemala City, GT, 01016. e-mail: estuardo.lira@usda.gov

Background: The Mediterranean fruit fly (*Ceratitidis capitata* (Wied.)) has posed a persistent threat to horticultural production, trade, and food security in the US, Mexico and Guatemala since its detection in the region. The Medfly Program officially began operations in Guatemala in 1975, establishing a national surveillance trapping network to monitor pest distribution and population dynamics. In 2004, the Program incorporated Geographic Information Systems (GIS) to georeference trap locations and integrate environmental variables, including temperature, rainfall, soil texture, and coffee-growing areas. Data generated between 2004 and 2007 supported the implementation of the Gradual Advance Plan (GAP), a cost-effective eradication strategy, and enabled early analyses linking trap captures with the El Niño–Southern Oscillation (ENSO). However, during 2019–2020, Medfly populations reached historically high levels, resulting from a convergence of favourable climatic conditions and strategic decisions that were later reassessed.

Methods: Species Distribution Modelling (SDM) techniques were applied using Medfly surveillance data collected between 2004 and 2020. Generalized Linear Models (GLMs) were developed in R to evaluate the relationship between Medfly abundance and environmental drivers, as well as the mitigating effects of two primary control interventions: the Sterile Insect Technique (SIT) and bait spray applications. Model outputs were translated into spatial risk maps depicting the probability of Medfly occurrence across habitats and altitudinal gradients in Guatemala. In parallel, population trends were analysed using analogue years to forecast potential population trajectories under varying climatic and operational scenarios.

Results: The modelling framework clarified the relative influence of environmental conditions and control interventions on Medfly population dynamics. While higher temperatures were associated with increased population pressure, sustained area-wide application of the Sterile Insect Technique (SIT), complemented by timely bait sprays, effectively suppressed populations. The use of predictive modelling supported targeted deployment of control measures, improved resource allocation, and enhanced the program's capacity to respond to climatic variability within an area-wide integrated pest management framework.

Conclusions: Integration of long-term surveillance data with species distribution modelling strengthened strategic planning and operational decision-making within the Medfly Program. Model outputs informed strategic adjustments, supported funding decisions, and guided operational prioritization, contributing to the reversal of the 2019–2020 outbreak and the reduction of Medfly populations to levels comparable to 2015 by 2025.

Key words: *Ceratitidis capitata*, Species Distribution Modelling, Sterile Insect Technique, Integrated Pest Management

FIELD EVALUATION OF A STERILE INSECT TECHNIQUE PROGRAM AGAINST *CERATITIS CAPITATA* IN TÜRKİYE

Ümran Akkan Demirer¹, Ferit Turanlı², Tevfik Turanlı¹, Özge Helvacıoğlu¹, İsmet Acar¹ & Hasibe Altıntaş¹

¹ Bornova Plant Protection Research Institute, Bornova, İzmir, Türkiye

² Department of Plant Protection, Faculty of Agriculture, Ege University, İzmir, Türkiye,

E-mail: umran.akkan@tarimorman.gov.tr

Background: The Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), is a major pest causing significant economic losses in fruit production worldwide. In Türkiye, increasing population density and distribution have made sustainable alternatives to chemical control essential. The Sterile Insect Technique (SIT) has been implemented as an effective environmentally friendly tool within area-wide integrated pest management programmes.

Methods: This study presents the field performance of a SIT release program conducted in Türkiye. A genetic sexing strain (*C. capitata* Vienna 8D53-FDF) was introduced for the first time into Türkiye and used for male-only releases. Field trials were carried out from 2022 to 2024 in a 25-ha citrus area in Eğriliman (İzmir), with a nearby area under conventional management serving as the control. Sterile males were released regularly, and pest populations were monitored using flies per trap per day (FTD) and fruit sampling.

Results: Results showed substantial suppression of *C. capitata* populations in the treated area. By the end of 2024, FTD values were 0.10 in the release area and 8.96 in the control. Fruit infestation rates were 0.36% in the SIT area versus 9.10% in the control. Abbott formula calculations indicated progressive SIT efficacy of 57.30%, 89.13%, and 95.55% in 2022, 2023, and 2024, respectively.

Conclusions: These results demonstrate that SIT is an effective and environmentally sustainable method for suppressing *C. capitata* populations under field conditions in Türkiye. The findings support the integration of SIT into national IPM programmes, contributing to reduced pesticide use, improved export compliance and the promotion of sustainable agriculture in line with European Green Deal objectives.

Key words: Mediterranean fruit fly, Pest suppression, Citrus, Fruit infestation, Sustainable agriculture

Improving Fruit Fly Program Efficiency with Science-Based Analysis

Corinna S. Bazelet, Kevin Bigsby, Mathias Marcos, Yu Takeuchi, Thom Worm,
Amber Tripodi

United States Department of Agriculture, Animal and Plant Health Inspection Service,
Plant Protection and Quarantine (USDA APHIS PPQ), 5601 Sunnyside Ave.
Beltsville, MD 20705, USA. E-mail: corinna.s.bazelet@usda.gov

Background: Exotic fruit flies threaten over 400 different types of plants and a \$14.8 billion agricultural industry in the United States. Fruit fly programs in the United States, overseen by USDA, focus on pest exclusion, early detection, and rapid response to eradicate outbreaks and prevent exotic fruit flies from establishing in the U.S. As invasion risks and costs climb, USDA continuously works to improve the efficiency and effectiveness of fruit fly program operations. Science-based analysis plays a key role in efficiency improvements.

Methods: During 2025, analysts in USDA APHIS PPQ led the effort to address three major challenges facing the fruit fly program: (1) to reduce costs associated with sterile insect releases by evaluating the quantity and locations of sterile Medfly (*Ceratitis capitata*) and Mexfly (*Anastrepha ludens*) releases; (2) to improve trapping efficiency by evaluating the densities and types of traps and lures used across the trapping array; and (3) to standardize quarantine management by developing a new tool to more accurately estimate when to declare eradication of an outbreak.

Results: Altogether, these analyses led to significant program improvements across several operational activities without increasing pest risk. Some of these improvements included reduced costs associated with routine, preventive sterile insect releases and surveillance, alleviating lure supply challenges caused by production bottlenecks, and reducing variability in how quarantine durations are determined.

Conclusions: Through these initiatives, USDA APHIS demonstrates its commitment to safeguarding U.S. agriculture by applying rigorous science to practical challenges. Our work in 2025 helped fruit fly programs achieve significant cost savings, improve operational resilience, and strengthen confidence in pest management decisions.

Key words: Mediterranean fruit fly, Mexican fruit fly, sterile insect technique, eradication

INNOVATIVE STRATEGIES IN *CERATITIS CAPITATA* CONTROL: THE MOROCCAN STERILE INSECT TECHNIQUE PROGRAM

Amine Leghlid, Yahya Machal, Rayhanna Adnasse & Hassan Zouhry

Sterile Insect Unit, Maroc Citrus, Agadir, Morocco. E-mail: Amineleghlid2@gmail.com

Background: Tephritid fruit flies are among the most destructive pests of horticultural crops worldwide. In Morocco, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), represents a major constraint to the citrus industry, leading to yield losses, increased production costs, quarantine restrictions, and environmental concerns related to pesticide use. Stricter maximum residue limits imposed by international markets, particularly the European Union, have intensified the need for sustainable alternatives.

Methods: Morocco has adopted an area-wide integrated pest management strategy centered on the Sterile Insect Technique (SIT). With technical support from the FAO and the IAEA, a mass-rearing, irradiation, and release facility was established near Agadir. The program integrates mass production of sterile male flies, aerial releases, population monitoring, quality control, and a national surveillance network for early detection of invasive fruit fly species. The facility is designed to produce up to 130 million sterile male *Ceratitis capitata* per week, covering approximately 60,000 hectares of citrus-producing areas. Sequential releases are being implemented through defined operational blocks, supported by GIS-based decision-making and in line with FAO/IAEA international standards.

Results: During the implementation phase, regular releases of sterile male *Ceratitis capitata* were carried out over the treated areas. In non-treated areas, medfly populations remained relatively high, as indicated by elevated flies per trap per day (FTD) values. In contrast, treated areas showed a clear reduction in medfly populations following the initiation of sterile male releases. Both FTD values and fruit infestation levels decreased progressively after treatment, demonstrating a suppression of the wild population associated with the SIT implementation. Laboratory quality control assessments confirmed the good biological quality of the sterile males produced, with satisfactory levels of sterility, emergence, flight ability, and competitiveness. These parameters met international quality standards and supported the effectiveness of field releases.

Conclusions: The SIT program represents a major step toward sustainable area-wide fruit fly management in Morocco. It is expected to significantly reduce pest pressure, minimize pesticide use, improve fruit quality, and safeguard market access for Moroccan citrus.

Key words: *Ceratitis capitata*, Sterile Insect Technique, citrus, Morocco, area-wide IPM

Mobile Modular Packing Centers for Multi-Species Sterile Insect Technique Programs: Operational Flexibility, Cost Optimization, and Quality Assurance

José Luis Zavala López¹, Rene Cano Pérez¹, Armando Yada Leal¹

¹ Servicios Mubarqui, México (jlzavalalopez@gmail.com)

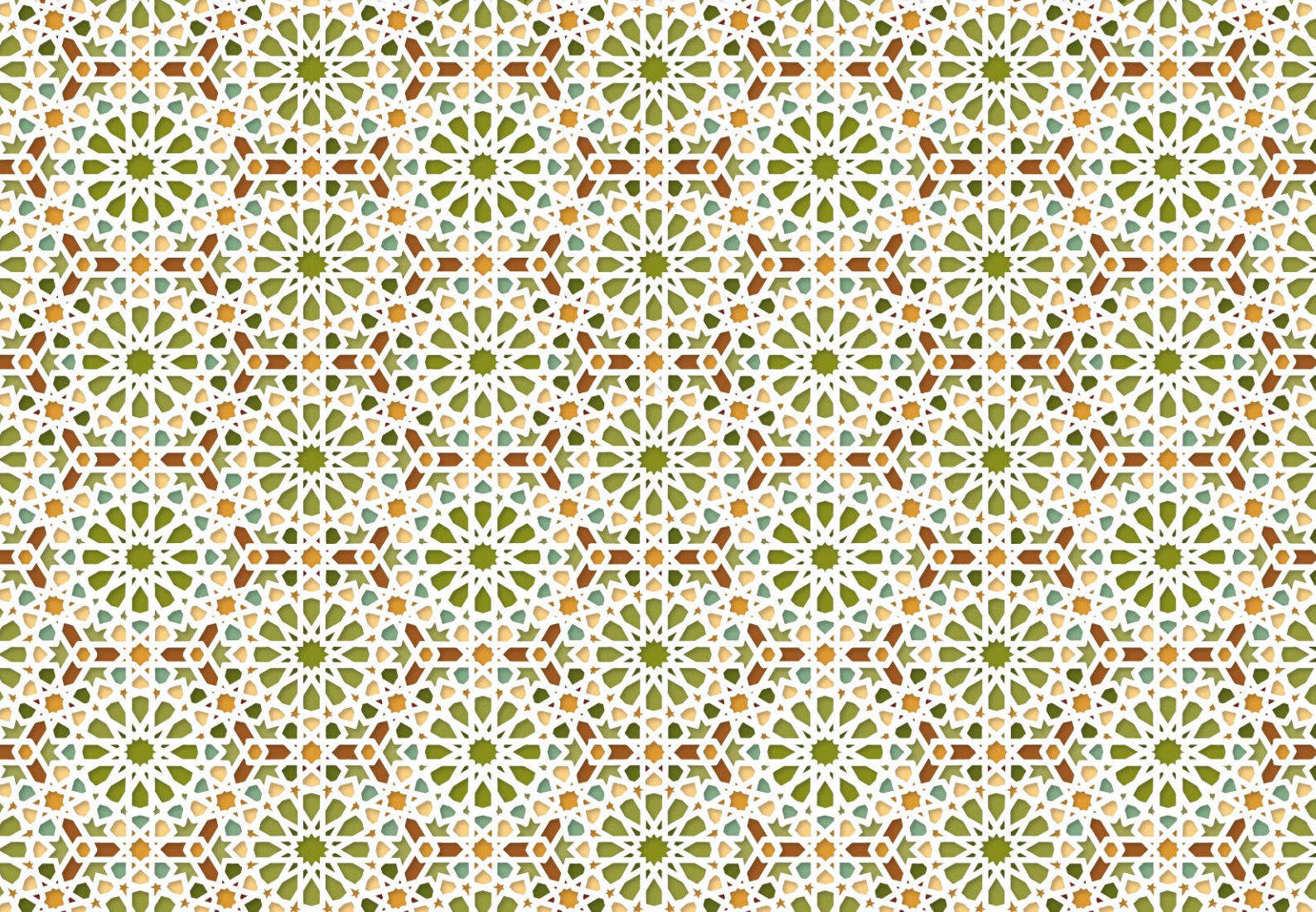
Background: The implementation of the Sterile Insect Technique (SIT) requires efficient and adaptable infrastructure for the handling and preparation of sterile insects. Conventional fixed facilities often involve high capital investment, extended installation periods, and limited flexibility in response to changing operational needs. The design, implementation, and evaluation of a mobile modular packing center has been developed to support multi-species SIT programs, including *Ceratitis capitata* (Mediterranean fruit fly), *Anastrepha ludens*, (Mexican fruit fly), *Diachasmimorpha longicaudata* (Parasitoids) and *Cochliomyia hominivorax* (New World screwworm).

Methods: The system consists of transportable modules integrating controlled environmental conditions (temperature, humidity, and light), optimized workflow distribution, and standardized equipment tailored to operational requirements. Its modular nature allows rapid relocation and deployment without the need for permanent infrastructure, significantly reducing capital investment and implementation time. A field application for *Anastrepha ludens* was conducted in Tapachula, Chiapas, Mexico, under a coordinated program involving the National Fruit Flies Program and local mango producers' organizations. This implementation demonstrated the system's capability to operate effectively under real production conditions, providing logistical flexibility and proximity to target areas.

Results: Quality control results showed consistently high biological performance, with emergence rates ranging from 93% to 97%, and flight ability values exceeding the minimum thresholds established by operational standards. These results confirm that the modular system maintains, and in some cases surpasses, the biological quality achieved in conventional facilities. While initial cost reductions were not significant during the first phase, substantial economic advantages were realized in subsequent implementations. The modular approach eliminated the need for permanent infrastructure in new operational sites, resulting in considerable savings in capital investment and enabling faster program expansion.

Conclusions: The results confirm that mobile modular packing centers are a robust, flexible, and cost-effective solution for SIT programs, particularly in scenarios requiring multi-species management, rapid deployment, and scalable operations without compromising biological quality.

Key words: Mediterranean fruit fly, Mexican fruit fly, sterile insect technique, New World Screwworm, parasitoids



ORAL COMMUNICATIONS

Social, Economic and Policy Issues of Action Programs

REUSE OF SPENT LARVAL DIET TO IMPROVE THE ECONOMIC AND ENVIRONMENTAL SUSTAINABILITY OF SIT PROGRAMS

Beatriz Turinetto, Sergio Ramírez, Lucia Rodríguez, Eduardo Vallejo, Cynthia McCormick, Juan Asfennato, José Orts

Institute of Agricultural Health and Quality of Mendoza (ISCAMEN), Mendoza, Argentina.

E-mail: beatrizturi@gmail.com

Background: The Sterile Insect Technique (SIT) is a key component of Integrated Pest Management (IPM) for fruit flies; however, its economic sustainability strongly depends on reducing mass-rearing production costs, particularly those associated with larval diet ingredients, which historically account for approximately 38% of total costs, under the *ceteris paribus* assumption that all other economic variables remain constant.

Methods: This study evaluates a methodology for the recovery, sanitization, and reuse of spent larval diet at ISCAMEN. Residual diet was treated through steam extrusion at 85 °C to ensure elimination of biological residues and substrate sanitization. The processed material was reincorporated as a partial bulking agent, replacing 50–70% of corn cob in new diet formulations. Multiple formulations were tested to achieve appropriate texture and nutritional balance through structural adjustments and targeted nutrient supplementation.

Results: Biological quality control parameters —pupal weight, adult emergence, proportion of fliers, and flight ability— remained within FAO/IAEA Manual standards, with no statistically significant differences compared to the control diet.

Conclusions: Reuse of the processed substrate resulted in a substantial reduction in diet production cost, on the order of 28% to 39% of the larval diet cost, improved operational efficiency, and decreased agroindustrial waste generation, supporting the economic and environmental sustainability of SIT-based fruit fly control programs.

Key words: Sterile Insect Technique; fruit flies; mass rearing; larval diet; spent diet reuse; cost efficiency; sustainability

Session: Social, Economic and Policy Issues of Action Programs

Navigating the Policy Dimensions of Sterile Insect Technique Application in Europe: Insights from the REACT Project

Thomas Stollenwerk, Michael Anranter

Oikoplus GmbH, Millergasse 37/3, 1060 Vienna, Austria. E-mail: stollenwerk@oikoplus.com

Background: The Sterile Insect Technique (SIT) has demonstrated operational feasibility in several global settings. However, translating SIT into scalable field applications remains challenging. In Europe, regulatory uncertainty affects planning, mass-rearing design, risk communication, and the long-term sustainability of action programmes. Beyond technical optimisation, practitioners must navigate administrative procedures, societal expectations, and cross-sector coordination. In particular, the use of genetic methods, such as those that could be used in the development of genetic sexing strains to increase the efficiency of SIT applications, faces social and regulatory hurdles, especially in Europe. REACT examines how these policy and governance factors directly influence the practical implementation of SIT in Europe.

Methods: Using insights from expert interviews, document analysis, and participant observation within SIT research and innovation projects, the study identifies recurring policy bottlenecks and operational challenges experienced by scientists, regulators, and programme managers. This approach highlights how regulatory interpretations, communication practices, and institutional roles shape real-world deployment conditions.

Results: Preliminary findings indicate that SIT is understood through competing lines of argumentation and narratives: as an ecological and operational innovation, as a regulatory challenge, and as a biotechnology closely associated with public and political sensitivities around genetic modification. These different framings influence how feasibility and risks are assessed in practice. While research communities develop shared technical expectations and operational standards, these internal alignments do neither automatically translate into wider institutional or societal acceptance nor into shared business models building on a joint regulatory framework. One key source of uncertainty concerns the regulatory status of CRISPR-based genetic sexing strains. Divergent interpretations of EU GMO legislation create a mismatch between emerging technological capabilities and the current governance framework.

Conclusions: Policy and governance factors significantly shape the operational viability of SIT programmes in Europe and beyond. Enhancing clarity in regulatory procedures, improving dialogue between scientific and administrative actors, and aligning public communication strategies with field realities could lower barriers to SIT adoption. Insights from REACT demonstrate that successful SIT deployment depends not only on biological performance but equally on anticipatory governance, regulatory preparedness, and stakeholder alignment. These findings offer practical guidance for future SIT action programmes and for designing pathways that enable emerging sex-separation technologies to reach applied use.

Key words: Sterile Insect Technique, policy implementation, regulatory frameworks, mass rearing, CRISPR-based genetic sexing, REACT project

Abir Hafsi, Laura Moquet, Michael Ben-Yosef, Marc De Meyer, Nikos T. Papadopoulos, Vasilis G. Rodovitis, Pierre-François Duyck, Aruna Manrakhan, Ana Larcher Carvalho & H  l  ne Delatte

Background: Biological invasions by fruit flies (Diptera: Tephritidae) represent a major ecological and economic concern worldwide, yet their impacts on resident tephritid communities remain fragmented across empirical studies. We conducted a meta-analysis to quantitatively assess the global ecological impacts of *Bactrocera dorsalis* and *Bactrocera zonata* invasion on resident communities, at species, population, community and ecosystem levels.

Results: Overall, invasive *B. dorsalis* and *B. zonata* exerted significant negative effects on resident tephritid communities, leading to reduced abundance of native fruit fly species fitness, and increased fruit losses. It seems that invasion of both *Bactrocera* species impacted fitness and physiology of the host plant. The magnitude of effects varied depending on functional characteristics of the resident species. Our findings highlight the context-dependent nature of *B. dorsalis* and *B. zonata* invasions and emphasize their capacity to restructure resident communities beyond direct competitive effects.

Key words: Invasion, fruit fly, competition, effect size, displacement, host plant, resident species

Unraveling environmental drivers of insect pest dynamics across multiple landscapes

Cécile Caumette^{1,2}, Marie-Pierre Chapuis^{1,2}, Sylvain Piry², Thierry Brévault^{3,4,5}, Ousmane Ndoye⁶, Aristide Diatta⁶, Julien Papaïx⁷, **Karine Berthier**^{2,8}

¹ CIRAD, CBGP, Montpellier, France

² CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, University of Montpellier, Montpellier, France

³ CIRAD, UPR AIDA, Biopass, Centre de Recherche ISRA-IRD, Dakar, Senegal

⁴ AIDA, Univ Montpellier, CIRAD, Montpellier, France

⁵ CIRAD, UPR AIDA, icipe, Duduville Campus, Nairobi, Kenya

⁶ BIOPASS, IRD-CBGP, ISRA, UCAD, Campus de Bel-Air, BP 1386, CP 18524 Dakar, Senegal

⁷ INRAE, BioSP, Avignon, France

⁸ INRAE, Pathologie Végétale, Montfavet, France

E-mail: karine.berthier@inrae.fr

Background: The persistence of pest populations during crop off-seasons is a major challenge for integrated pest management, yet the ecological processes underlying survival and re-infestation often remain unclear.

Methods: We investigated the annual population dynamics of the oriental fruit fly, *Bactrocera dorsalis*, a major mango pest, based on a two-year demographic monitoring conducted across 56 orchards in Senegal, spanning the Sahelian Niayes and the tropical Casamance. Using a framework combining mechanistic and machine learning models, we identified the main environmental drivers of two key demographic parameters: the local abundance during the mango off-season and the onset of population growth in orchards.

Results: Dynamics differed markedly across regions. In the Niayes, harsh dry-season conditions confined residual populations to microhabitats providing humidity and shade, while alternative hosts such as citrus trees acted as catalysts for early outbreaks. In contrast, under tropical conditions in the Casamance basin, *B. dorsalis* populations were more abundant year-round, and higher off-season densities appeared to delay outbreak onset, likely due to density-dependent processes such as competition and predation.

Conclusion: These findings highlight how distinct ecological processes shape *B. dorsalis* persistence across contrasting environments and suggest that management strategies should be region-specific: targeting refuges and early host resources in the Niayes, while in Casamance, focusing on enhancing natural regulation may be more effective in reducing pest pressure.

Key words: *Bactrocera dorsalis*, population dynamics, abundance time series, machine learning, weather, landscape, agricultural practices, crop off-season

Session 1. Biology, Ecology, Physiology and Behavior

The role of nearby backyard fruit gardens on medfly development

Marco Colacci^{1,2}, Andrea Sciarretta¹, Valeria Papili¹, Ennio Vallillo¹, Nicola Mori³, Slawomir A. Lux²

¹Department of Agricultural, Environmental and Food Sciences, University of Molise, Via de Sanctis, Campobasso, Italy

²inSilico-IPM, PL-05-510 Konstancin-Jeziorna, Poland

³Department of Biotechnology, University of Verona, San Floriano (Verona), Italy

E-mail: marco.colacci@unimol.it

Background: *Ceratitis capitata* (medfly), is one of the most destructive pests of fruit crops worldwide. The influence of host resources in the environment surrounding fruit orchards on the development and maintenance of medfly populations is widely recognized. The role of home gardens is particularly important in medfly suppression efforts, as well as in “standard” farm-level IPM practices implemented in mosaic landscapes. However, regardless of their generally recognized importance, the actual impact of such “supporting resources” in specific local conditions is difficult to assess and quantify. Here, we present simulation of medfly influx from nearby resources considering varying distances and host structure between source and sink niches.

Methods: All simulations were performed using the PESTonFARM software. The model simulates lifetime development, dispersal and fate of large cohorts of individual medfly females according to the local farm topography, site-specific weather patterns, fruit distribution and phenology, and a combination of IPM treatments. It stochastically simulates the daily behaviour of each individual female, its oviposition choices and fruit infestation events, its local dispersal and shifts among plots with various fruit species and cultivars, and mortality events triggered by natural causes and/or assumed IPM treatments. The model operates at 10x10m spatial and daily temporal resolution. Several scenarios were simulated in real farms located in Central-Italy to show the role of home gardens, which grow a variety of fruits present at different times of the year, on medfly development on a nearby farm, which is deprived of early fruits, which are crucial for the year-round survival of local medfly population.

Results: Some simulations, taking into account the presence of the backyard resources, showed that with a random distribution of the same number of females, a few individuals overwintering in the backyard gardens built up local populations there during the spring, after which some of their offspring migrated to the nearby farm and caused a significant increase in the medfly population even at the lowest initial density. Other simulations show the significant impact of nearby backyard gardens on the medfly population and fruit infestation in the farm.

Conclusions: Our simulations revealed and quantified significant medfly movement between nearby niches, even when separated by distances of several hundred meters. At a mean range of about 250 meters, seasonal influxes of medfly can be substantial, temporally variable and asymmetric between locations with different fruit host profiles, sufficient to support significant medfly populations on affected farms and cause severe infestation of both mid-season and late fruits.

Key words: Mediterrean Fruit Fly, PESTonFARM, simulation, dispersal

Host use and phylogeny of African *Dacus* (Diptera: Tephritidae) inferred from genome-scale data

Lore Esselens¹, Domingos Cugala², Maulid Mwatawala³,
Massimiliano Virgilio¹, Marc De Meyer¹

¹Royal Museum for Central Africa (RMCA), Tervuren, Belgium; ²Eduardo Mondlane University (EMU), Maputo, Mozambique; ³Sokoine University of Agriculture (SUA), Morogoro, Tanzania. E-mail: lore.esselens@africamuseum.be

Background: The genus *Dacus* Fabricius, 1805 (Diptera: Tephritidae) comprises more than 270 species, most of which occur in the Afrotropical region and include several economically important agricultural pests. The phylogenetic relationships within *Dacus* and the evolutionary history of host plant specialization remain incompletely resolved. Previous Sanger-based phylogenies suggested an association between host plant use and phylogenetic structure, but were constrained by limited marker resolution and taxon sampling. Here, we apply genome-scale data to reconstruct nuclear and mitochondrial phylogenies of African *Dacus* and to place these phylogenetic relationships in a broader evolutionary and temporal context.

Methods: We analysed 179 specimens representing 51 African *Dacus* species, a substantially expanded sampling compared to previous study (71 specimens from 32 species). Whole-genome sequencing was performed on both newly extracted material and long-term preserved DNA vouchers. Phylogenetic inference was based on orthologous groups identified using OMA standalone, with sequence reconstruction performed using the Read2Tree pipeline. Maximum-likelihood phylogenies were inferred from concatenated nuclear and mitochondrial nucleotide datasets using IQ-TREE2. Divergence times were estimated using a reduced set of conserved orthologous groups and fossil-calibrated molecular clock analyses implemented in MCMCtree.

Results: Genome-scale nuclear phylogenies recovered strongly supported clades corresponding to currently recognised *Dacus* species, providing robust validation of species boundaries and genus-level relationships. Major lineages showed consistent clustering by host plant family, with Apocynaceae-associated taxa forming a well-supported monophyletic group and Passifloraceae feeders nested within a broader paraphyletic group of Cucurbitaceae-associated species. Mitochondrial phylogenies were largely congruent with nuclear results, although with lower support and minor topological differences. The analyses revealed increased lineage structure within *Dacus humeralis*, with three distinct clusters instead of the two previously recognised. Fossil-calibrated divergence time estimates, incorporating representatives of major Dacini lineages, placed *Dacus* diversification within a broader temporal framework.

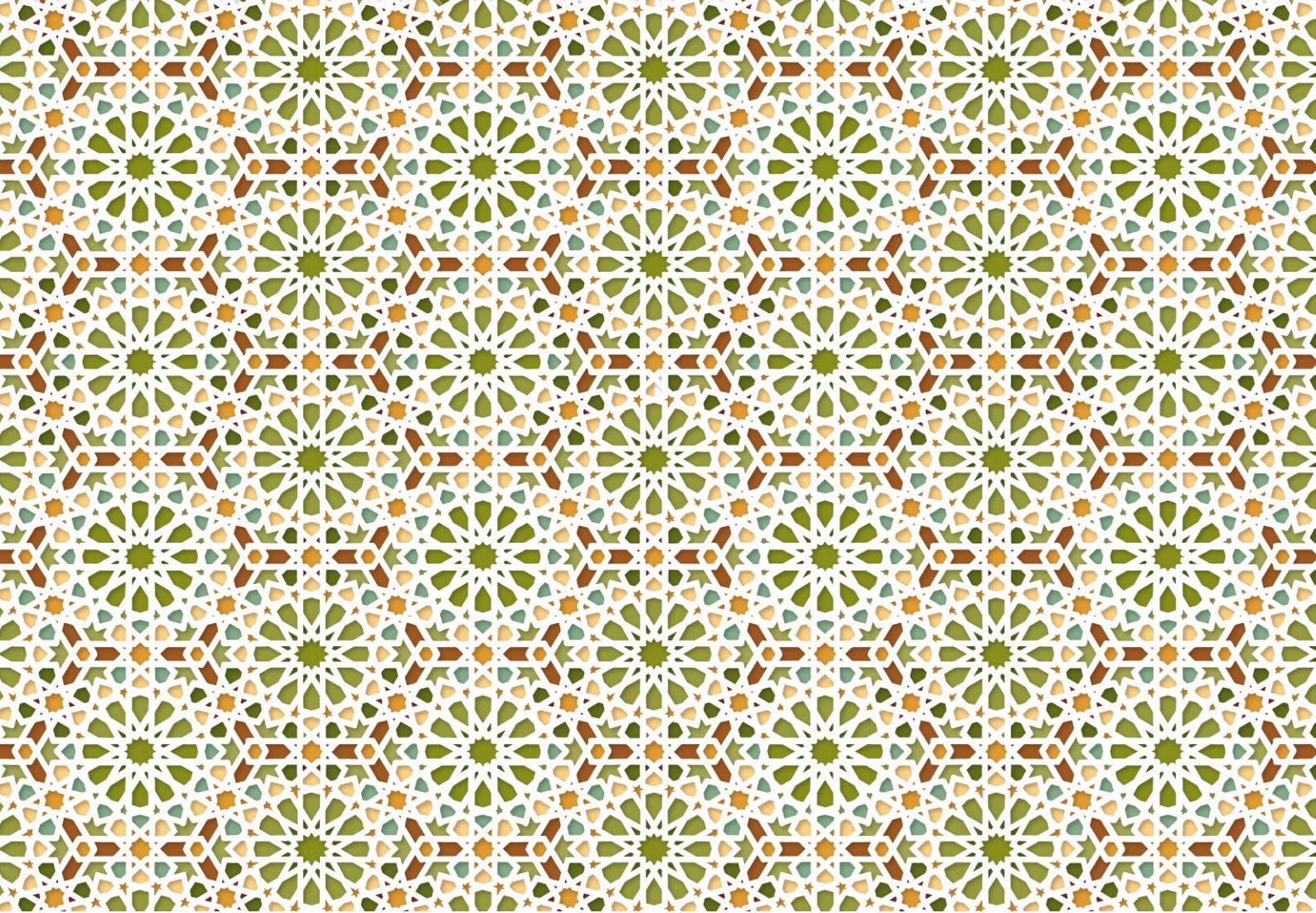
Conclusions: By combining expanded taxon sampling with genome-scale data, our study provides a robust and refined phylogenetic framework for African *Dacus*. The results support host-associated diversification, validate earlier phylogenetic hypotheses with substantially increased resolution, and reveal previously unrecognised lineage complexity within key taxa. These findings demonstrate the power of phylogenomics for resolving evolutionary relationships in species-rich insect groups and provide a foundation for future taxonomic, ecological, and pest-management studies.

Key words: true fruit flies, *Dacus*, phylogeny, orthologous genes

Session 3: Genetics and Biotechnology



POSTERS



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Biology, Ecology, Physiology and Behavior

Effect of photoperiod on thermal tolerance of *Ceratitis capitata* (Diptera: Tephritidae)

Antonis G. Papadopoulos & Nikos T. Papadopoulos

Laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Fytokou St., 384 46 Nea Ionia (Volos), Magnisia, Greece.

E-mail: antoniopap@uth.gr

Background: Thermal tolerance is central to determining the invasion potential, persistence, and climatic vulnerability of agricultural pests. However, few studies have examined how non-diapausing tropical species respond to photoperiod, an environmental cue that can modulate seasonal physiology independently of temperature. This study evaluates, for the first time, the influence of photoacclimation on thermal tolerance in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), a globally significant agricultural pest.

Methods: Two laboratory experiments were conducted to assess critical thermal limits (CT_{min} and CT_{max}). In Experiment 1, individuals were reared from egg to adult under four photoperiods (0:24, 8:16, 14:10, and 24:0 L:D) under controlled conditions (25±1°C, 55±10% RH, 1000±200 lux). In Experiment 2, all immature stages developed under a long-day photoperiod (14:10 L:D). Upon emergence, adults of both experiments experienced the above four photoperiods for 10 days. Thermal tolerance was measured on day 11 of adult life.

Results: Developmental photoperiod had a pronounced effect on both critical thermal limits. Long-day conditions resulted in the lowest CT_{min}, whereas short-day rearing resulted in the highest. The highest CT_{max} values occurred in individuals reared under constant darkness, particularly in females. When the different photoperiod treatments were applied only during adulthood, CT_{min} showed no significant change; however, CT_{max} was markedly reduced in flies maintained under constant light compared with other treatments.

Conclusions: Photoperiod alone, independent of temperature, can drive thermal tolerance in *C. capitata*, with developmental exposure exerting stronger effects than adult-only photoacclimation. These findings emphasize the importance of integrating photoperiodic cues into ecological models of thermal physiology and invasion success, ultimately improving the realism of pest risk assessments and the development of climate-resilient management strategies.

Key words: medfly, photoacclimation, early life experience, adult performance, CT_{min}, CT_{max}, thermal biology, photobiology

PATTERNED WINGS OF *CERATITIS COSYRA* DO NOT DETER PREDATION BY *THYENE NATALI*

Jessica Young¹, Tharina Bird^{1,2}, Gonzalo Iván Gonzalez-López¹, Marietjie Oosthuizen¹, **Christopher W. Weldon¹**

¹Department of Zoology and Entomology, University of Pretoria, Hatfield 0028, Pretoria, South Africa

²DITSONG National Museum of Natural History, Pretoria 0001, South Africa

E-mail: cwweldon@zoology.up.ac.za

Background: Fruit flies (Diptera: Tephritidae) rank among the most significant agricultural pest species globally, particularly in the fruit production sector. It is therefore of utmost importance to understand the behaviour of these flies for developing effective management strategies and mitigate crop losses. Many fruit fly species have complex wing patterns, which may have a role in predator avoidance. Some tephritid species have been proposed to mimic jumping spiders (Salticidae) through their wing patterns and display behaviours. This study investigated whether the patterned wings of marula fruit flies, *Ceratitis cosyra* (Walker, 1849), are used as a predator avoidance tactic against the gold banded jumping spider, *Thyene natali* G.W. Peckham and E.G. Peckham, 1903.

Methods: Ten male *T. natali* spiders were each tested against male and female *C. cosyra* flies that had received four wing treatments (normal, painted, patterned, enhanced), encountering all eight combinations in random order. Interactions were filmed for 5 minutes in a 14-cm Petri dish. Videos were analysed in BORIS and ImageJ to quantify spider opisthosoma and fly body length, latency of attack, attack distance, and the frequency of successful attacks, failed attacks, stalking, foreleg displays, abdomen displays, supination, and abandonment of approaches by spiders.

Results: The natural wing patterns of *C. cosyra* had no significant effect on hunting behaviour by the spiders. Spiders showed no reduction in attack success or increased hesitancy toward flies with normal or enhanced treatments compared to those with pattern and painted treatments. Instead, spider behaviour was more strongly influenced by prey size and sex.

Conclusions: These findings suggest that *T. natali* bases attack decisions primarily on prey movement, shape, or size, rather than wing patterning.

Key words: Mimicry, jumping spider, predator avoidance, predator-prey interactions

Plant-derived sterol promotes superior *Bactrocera dorsalis* immature development over cholesterol in a holidic diet

G. I. González-López¹, C. Ruth Archer², John S. Terblanche³, **Christopher W. Weldon¹**

¹Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

²Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Germany

³Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, South Africa

E-mail: cwweldon@zoology.up.ac.za

Background: Fruit flies (Diptera: Tephritidae) include several species that are major agricultural pests worldwide. Sustainable management often relies on the Sterile Insect Technique (SIT), whose success depends on the biological quality of released males, which is directly influenced by the larval diet. These diets are commonly yeast-based or provide cholesterol as a sterol source. Since insects cannot synthesise sterols, their dietary composition is critical for growth, metamorphosis, and reproduction. However, the contribution of alternative sterols in fruit fly development remains poorly understood. We used *Bactrocera dorsalis* (Hendel) to evaluate sterol effects on immature development, and tested egg dechoriation to explore the role of egg-associated bacterial communities in this relationship.

Methods: A holidic larval diet developed for *Drosophila melanogaster* was modified for this study. Five diets were prepared, each containing a single steroid chemical (cholesterol, cholestan-3-one, ergosterol, sitosterol, or stigmasterol) at a standard concentration. These were compared to a standard diet based on brewer's yeast and carrot powder. For each replicate (n = 10), diets were inoculated either with eggs sterilised by dechoriation or intact eggs. Survival, development time, and pupal weight were recorded.

Results: Sterol type significantly influenced immature development. Sitosterol promoted higher survival, faster development, and heavier pupae compared with cholesterol. Ergosterol and cholestan-3-one increased larval mortality and reduced adult emergence. Cholesterol and stigmasterol supported development but with lower performance than sitosterol. Egg dechoriation generally increased mortality and delayed development, while effects on pupal weight and adult emergence were more variable and sterol-dependent, suggesting interactions between sterol type and bacteria associated with eggs.

Conclusion: Our findings suggest that sitosterol is more compatible than cholesterol with the physiological needs of *B. dorsalis* larvae. This underscores the importance of plant sterols for optimising larval diets, offering a practical approach to strengthen mass rearing for the SIT.

Key words: Larval diet, sterols, microbiota, mass rearing, sterile insect technique (SIT)

Monitoring of Spotted Wing *Drosophila* in blackberry crops in southern Romania

Constantina Chireceanu¹, Maria Iamandei¹, Andrei Teodoru¹, Ionuț Popa¹,
Madalina Radulea¹ & Damian Dragomir²

¹Research and Development Institute for Plant Protection, 8 Ion Ionescu de la Brad, 013813, Bucharest, Romania

²Research and Development Station for Fruit Trees Growing Baneasa, 4 Ion Ionescu de la Brad, 013813, Bucharest, Romania

E-mail: cchireceanu@yahoo.com

Background: *Drosophila suzukii* (Diptera, Drosophilidae) known as the Spotted Wing *Drosophila* (SWD) is an invasive vinegar fly native to the Asian continent, which entered Europe in 2008 from where it spread in many countries; in Romania SWD was detected in 2013. Healthy soft skinned fruits such as raspberries, blackberries, blueberries, grapes, cherries are among the most susceptible to its attack. Due to the high demand for fresh local fruits, blackberry cultivation, like other small berry crops, has seen a significant increase in the cultivated area in Romania in recent years. At the same time, the blackberry crops are among the target crops for SWD that develops established populations in many areas in the country. The paper presents results on the dynamics of SWD adults in four cultivated blackberry plots located in southern Romania, carried out in 2024 and 2025.

Methods: Adult population of *D. suzukii* was monitored using plastic bottles (0.5ml) baited with a mixture of apple cider vinegar and red wine, from July to December of each monitoring year.

Results: SWD was trapped from July to November in 2024 and to December in 2025; the highest fly captures were during November following a decrease in atmospheric temperatures, coinciding with a period when a small number of fruits were damaged. A fluctuation in the sex ratio of males per females was observed during the monitoring period.

Conclusions: Although SWD adult captures have shown variable populations from one crop to another and from one year to another according to climatic conditions, the threat to blackberries exists and is likely to increase in the coming years.

Key words: adult population dynamics, Spotted Wing *Drosophila*, blackberry, Romania

Host plant and geographic effects on wing shape variation in *Drosophila suzukii* from Serbia

Boško Jezerkić¹, Hugo A. Benitez², Ivana Pajač Živković³, Helena Viric Gasparic³ & Darija Lemic³

¹ Forecasting and Reporting Service for Plant Protection of the Republic of Serbia, 21000 Novi Sad, Serbia

² Instituto One Health, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile

³ University of Zagreb Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia. E-mail: dlemic@agr.hr

Background: The spotted wing drosophila (*Drosophila suzukii*) is a major global pest of berry and stone fruits because it lays eggs in healthy, ripening fruit and has high reproductive potential, broad host range, and strong phenotypic plasticity. In Serbia, it is widely distributed across multiple hosts and regions, but the extent to which host use and geography shape wing morphology remains unclear.

Methods: Adults were sampled from seven Serbian localities across country (Čačak, Kraljevo, Leskovac, Niš, Novi Sad, Požarevac, and Ruma) and five host plants (blackberry, cherry, grape, peach, and raspberry). For each locality × host combination, 20–40 individuals (males and females) were analysed, using both left and right wings; in total, 860 wings were analysed. Wing shape was analysed using standard geometric morphometrics (landmark digitisation, generalized Procrustes analysis), followed by PCA and CVA. Group divergence was quantified with Procrustes distances and tested using Procrustes ANOVA with residual randomization.

Results: PCA showed strong overlap among individuals across hosts and localities (PC1 = 59.21%, PC2 = 18.40%), indicating no distinct clustering. CVA revealed partial separation among groups, and deformation patterns were concentrated mainly in distal/apical and posterior wing regions. Procrustes ANOVA detected significant effects of locality ($R^2 = 0.0346$, $p = 0.0001$), host plant ($R^2 = 0.0167$, $p = 0.0091$), and their interaction ($R^2 = 0.0310$, $p = 0.0010$).

Conclusions: Serbian *D. suzukii* populations exhibit subtle but significant wing shape differences linked to both geographic origin and host plant, with a significant host × locality interaction suggesting that host-associated variation depends on local environmental context. Although effect sizes were small and morphospace overlap remained high, these patterns are consistent with fine-scale phenotypic structuring in a highly dispersive, plastic invasive species. Wing geometric morphometrics therefore provides a sensitive framework to complement ecological monitoring and to support understanding of population variability relevant for pest management in Serbian agroecosystems.

Key words: spotted wing drosophila, geometric morphometrics, wing shape, host plant, phenotypic plasticity

Pest fruit fly (Diptera: Tephritidae) population fluctuations in a mixed production landscape in North West province, South Africa

Dylan Pullock¹, Aruna Manrakhan^{2,3}, Gulu Bekker³ & Christopher Weldon¹

¹Department of Zoology and Entomology, Forestry and Agriculture Biotechnology Institute (FABI), University of Pretoria, South Africa

²Citrus Research International, Mbombela, South Africa

³Department of Conservation Ecology and Entomology, Stellenbosch University, South Africa

E-mail: dylan.pullock@fabi.up.ac.za

Background: The oriental fruit fly, *Bactrocera dorsalis* (Hendel), and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) are known pests of some commercial citrus types in South Africa but also have several other host species that may be present in a mixed production region. They can cause direct damage resulting in yield loss, their presence in a region implies costs for their control to prevent infestation, and exports can be negatively impacted in case of detection in fruit. To optimise resources for regional pest control, identification and targeting of primary sources of pest population is required through evidence on their seasonal spatial distribution and abundance. The aim of this work is to define locations and times of the year when targeted fruit fly control measures can be applied to minimise infestation of citrus in a mixed production landscape. Part of this involves determining the pest fly species abundance over space and time.

Methods: In a mixed production landscape in the North West province of South Africa, 240 yellow bucket traps were deployed in pairs. One trap in each pair was baited with E.G.O. Pherolure (Insect Science, Tzaneen, Limpopo, South Africa), with the lure changed every 20 weeks. The other trap was baited with methyl eugenol (INVADER-LURE, River Bioscience, Gqeberha, Eastern Cape, South Africa), with the lure changed every 6 weeks. The paired traps were placed 40 metres apart, and these were located at least 100 metres from other trap pairs. Starting from July 2024, traps were emptied every 14 days and fruit flies caught were identified and recorded.

Results: The number of *C. capitata* caught per trap were relatively consistent but highly variable across the region throughout the year. The highest number of *C. capitata* per trap was in a nectarine orchard two to four weeks after the fruit had been harvested. Trap captures of *B. dorsalis* were low and spread out for most of the monitoring period but started to increase moving into January, specifically in traps present in citrus orchards.

Conclusions: Vegetation type appears to have an influence on the numbers of certain species caught in the traps. Nectarines may have acted as a source population for *C. capitata* in the region. The spike in *B. dorsalis* numbers in traps deployed in the eastern edge of the study region could be due to a more suitable microclimate provided by the citrus orchards there during that time of year. These results indicate that control measures for these pests should be focussed on their preferred vegetation types prior to peak of catches.

Key words: *Bactrocera dorsalis*, Oriental fruit fly, *Ceratitis capitata*, Mediterranean fruit fly, AWM

Effect of larval diet on adult longevity under starvation, desiccation, and non-stressful conditions in *Anastrepha fraterculus*

Fátima L. Fernández^{1,2,3}, Lucía Goane^{1,2}, Pilar Medina-Pereyra⁴, M. Rosario Jorge³, Diego F. Segura^{5,6}, Alejandra C. Scannapieco^{2,5}, David Nestel⁷, M. Teresa Vera^{1,2,8} & M. Josefina Ruiz^{1,2}

¹Facultad de Agronomía, Zootecnia y Veterinaria, UNT, Tucumán, Argentina; ²CONICET, Argentina; ³Facultad de Ciencias Naturales e IML, UNT, Tucumán, Argentina; ⁴Instituto de Fisiología Animal, Fundación Miguel Lillo, Tucumán, Argentina; ⁵Instituto de Genética (INTA), GV-IABIMO (CONICET), Hurlingham, Argentina; ⁶Instituto de Investigación en Ciencias Agrarias y Veterinarias, Universidad del Salvador, Argentina; ⁷Institute of Plant Protection, Department of Entomology, ARO, The Volcani Ctr., Beit Dagan 50250, Israel; ⁸Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, Vienna, Austria (current address). E-mail: flfernandez@csnat.unt.edu.ar

Background: Environmental stressors such as food deprivation and desiccation affect the survival of adult tephritid flies. Larval nutrition plays a key role in shaping adult traits related to stress tolerance. Understanding this role becomes relevant for improving mass-rearing in sterile insect technique (SIT) programs. *Anastrepha fraterculus*, a major target species of SIT, remains poorly studied in terms of tolerance to starvation and desiccation. This study evaluated the effect of larval diet on adult longevity of *A. fraterculus* under stressful and non-stressful conditions.

Methods: Three larval diets were evaluated, all based on non-hydrolysed brewer's yeast and sugar, but differing in additional ingredients and carbohydrate-to-protein ratios (C:P): 1) wheat germ and agar (C:P ca. 2:1); 2) corn flour (C:P ca. 3:1); and 3) wheat germ and mashed carrot (C:P ca. 5:1). Three hundred 48-hour-old eggs were inoculated into Petri dishes containing 58 g of one of the three diets. Starvation assays were applied to newly emerged adults, whereas desiccation assays were performed on newly emerged and 7-day-old adults. In addition, individuals were maintained under non-stressful conditions. Mean longevity was analysed using parametric models with Weibull distribution and Kaplan-Meier survival curves.

Results: Under starvation, longevity was highest in flies reared on the corn flour-based diet. Under desiccation stress, longevity was highest in flies reared on the corn flour- and wheat germ-based diets, in both newly emerged and 7-day-old adults. Under non-stressful conditions longevity was highest in adults reared on the carrot-based diet than in those reared on the wheat germ-based diet.

Conclusions: Larval diet composition influenced the survival of *A. fraterculus* adults under starvation and desiccation stress. The higher carbohydrate content of the corn flour-based diet could potentially favor the accumulation of energy reserves during larval development and, hence, allow for greater tolerance in adults. These findings suggest that optimizing larval diets may contribute to improved stress tolerance and longevity of mass-reared adults, thereby enhancing SIT results.

Key words: larval nutrition, South American fruit fly, stress, SIT programs.

REDEFINING THE ECOLOGICAL NICHES AND CLIMATIC DRIVERS OF *CERATITIS ROSA* AND *CERATITIS QUILICII* (DIPTERA: TEPHRITIDAE) USING ENSEMBLE MACHINE LEARNING

Jenipher Tairo¹, Joseph Ruboha¹, Happy Leonard^{1,3}, Ramadhani Majubwa¹, Abdul Kudra¹, Alcadro Barakabitze⁴, Maulid Mwatawala¹, Marc De Meyer², and Massimiliano Virgilio²,

¹Department of Crop Science and Horticulture, Sokoine University of Agriculture, Tanzania

² Biology Department, Royal Museum for Central Africa, Tervuren, Belgium

³Crop Protection Section, Tanzania Agricultural Research Institute (TARI), Morogoro, Tanzania

⁴Department of Informatics and Information Technology, Sokoine University of Agriculture, Tanzania

*Corresponding email for the first author: jenipher.tairo@sua.ac.tz

Background: *Ceratitis rosa* Karsch and *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio are cryptic species in the *Ceratitis* FARQ complex and a major threat to African horticulture. Yet their ecological requirements and continental-scale distribution remain poorly resolved. This study establishes their spatial distribution patterns and identifies the environmental and climatic drivers that influence their spatial distribution using machine-learning models.

Methods: Occurrence records were paired with high-resolution bioclimatic variables (Worldclim) to model habitat suitability. An ensemble of MaxEnt, BRT, and RF algorithms was employed to predict their potential distribution. Ecological niche divergence was assessed using PCA-env, and Schoener's *D* was applied to test the niche conservatism hypothesis.

Results: Ensemble predictions revealed that *C. rosa* is confined to the warm Indian Ocean coastal belt stretching from South Africa through Tanzania, to coastal Kenya, whereas *C. quilicii* dominates the temperate Cape in South Africa and the East African highlands. The models exhibit high predictive accuracy (AUC > 0.85), with temperature and elevation identified as the most important predictors of ecological segregation. Niche overlap was low (Schoener's *D* = 0.31), leading to the rejection of the niche equivalent hypothesis ($p < 0.05$). PCA-env indicated that divergence is mainly influenced by thermal gradients along PC1. Furthermore, Density preference curves revealed a broader thermal niche for *C. rosa* (23.69°C) compared to *C. quilicii* (18.09 °C).

Conclusions: This study provides the first continental-scale machine-learning distribution maps for *C. rosa* and *C. quilicii*, revealing clear ecological and geographic differences. These insights offer a robust framework for improving pest surveillance and phytosanitary risk assessment in Africa.

Keywords: *Ceratitis* FARQ complex, Machine Learning (ML), Species Distribution Models (SDMs), Pest management, Phytosanitary Risk Assessment.

EFFECT OF FRUIT COMPOUNDS ON THE SEXUAL COMPETITIVENESS OF STERILE *ANASTREPHA LUDENS* MALES

Luis A. Caravantes-Villatoro^{1*}, Julio C. Rojas¹, Ariane Dor¹, José S. Meza² & Pablo Liedo¹

¹El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km. 2.5, 30700. Tapachula, Chiapas, México. *Correspondence: luis.caravantes@estudianteposgrado.ecosur.mx

²Programa Operativo Moscas, SADER/SENASICA-IIICA, Camino a los Cacaotales S/N, 30860, Metapa de Domínguez, Chiapas, México.

Background: The Sterile Insect Technique (SIT) is currently used in Mexico to control pest populations of the Mexican fruit fly, *Anastrepha ludens* Loew. Similar to other SIT-controlled tephritid fruit fly species, it is essential that sterile *A. ludens* males remain sexually competitive. In this regard, several approaches have been investigated to enhance their sexual competitiveness, including post-tenal nutritional, hormonal, and semiochemical (aromatherapy) treatments. To our knowledge, the semiochemical treatments tested so far in *A. ludens* have been less successful compared with other tephritid species (e.g., *Ceratitis capitata* Wiedemann). In addition, the effectiveness of these treatments may depend on the nutritional status of sterile adults. In this work, we evaluated 1) the effects of aromatherapy with 3 different synthetic blends of volatile fruit compounds, and 2) the effect of adult feeding on fruit on the sexual competitiveness of sterile *A. ludens* males.

Methods: In the first experiment, sterile males of mass-reared *A. ludens* from the Tapachula-7 strain, fed on a 24:1 sugar-yeast diet, were exposed between days 4 and 8 after emergence, to a synthetic blend of volatile compounds derived from white sapote (*Casimiroa edulis*), bitter orange (*Citrus aurantium*), or guava (*Psidium guajava*). In the second experiment, adult males were fed on bitter orange or guava for 8 days after emergence. Control treatments included sterile and fertile wild males fed on a 24:1 sugar-yeast diet only. In the second experiment, a control treatment of sterile males fed on sugar only was also included. Sexual competitiveness was evaluated when sterile males reached 9 to 12 days, using field cages (3 x 2.1m) under natural conditions.

Results: No significant differences were observed in the percentage of matings obtained by males exposed to the synthetic blend of *Ca. edulis*, *Ci. aurantium* or *P. guajava* (Tukey, $p > 0.05$). However, males fed on *Ci. aurantium* achieved more matings than males fed on sugar only (Tukey, $p < 0.05$). This effect was not observed when comparing males fed on guava with males fed on sugar only (Dunn, $p > 0.05$).

Conclusions: The results obtained suggest that aromatherapy with a synthetic blend of volatile compounds from white sapote, bitter orange, or guava did not enhance the sexual competitiveness of sterile males of *A. ludens*. Interestingly, feeding sterile males with *Ci. aurantium* fruit increased the percentage of copulations, an effect that was not observed in males fed on guava. We discuss these results in terms of their potential applicability in the SIT programs for this species.

Key words: Mexican fruit fly, aromatherapy, semiochemical, Sterile Insect Technique, *Casimiroa edulis*.

Effect of experience on oviposition preference in females of *Anastrepha fraterculus* (Diptera: Tephritidae)

Garbalena, Micaela S.¹; Pacelli, Franco¹; Bachmann, Guillermo E.¹; Devescovi, Francisco¹; Nussenbaum, Ana L.^{1,2}; Ruiz, M. Josefina³; & Segura, Diego F.^{1,2}

¹ Instituto de Genética (INTA) – IABIMO (CONICET), Hurlingham, Buenos Aires, Argentina

² Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador, Buenos Aires, Argentina.

³ Facultad de Agronomía, Zootecnia y Veterinaria, Universidad Nacional de Tucumán, Argentina.

Background: Oviposition preference in *Anastrepha fraterculus* has been mainly studied in females reared on artificial diets and lacking prior experience, allowing the characterization of innate preferences only. Under these conditions, a strong preference for grapefruit over plum has been reported. In this study, we evaluated how experience during larval and adult stages may modify oviposition preference, aiming to assess the degree of behavioral plasticity in this species.

Methods: Three experiments were conducted. In the first experiment, adult females were exposed exclusively to either grapefruit or plum for 1, 3, or 7 days. In the second experiment, we evaluated the reversibility of experience-induced preference by testing females trained on plum after periods without fruit exposure. In the third experiment, the effect of the larval host was assessed by comparing the oviposition preferences of females reared on grapefruit or plum.

Results: Females with adult experience on plum did not show a preference for grapefruit, whereas those trained on grapefruit retained their original preference, indicating that adult experience can modulate oviposition substrate choice. Females trained on plum for seven days showed no preference after one day without fruit, but recovered their innate preference for grapefruit after three days, suggesting that the memory associated with acquired experience is transient. Females reared on plum showed no clear preference, while those reared on grapefruit maintained a strong preference for this fruit.

Conclusions: The results demonstrate that both larval and adult experience significantly influence oviposition site selection in *A. fraterculus*, revealing a high degree of behavioral plasticity. This plasticity has important ecological and applied implications, particularly for the development of behavior-based control strategies in integrated pest management programs.

Keywords: behavioral plasticity; memory; host plant; pest insect.

Abstract

Water deprivation resistance influenced by age, sex, and diet in *Bactrocera oleae*

Evangelia I. Balampekou¹, Dimitrios S. Koveos¹, Thomas M. Koutsos², Georgios C. Menexes³ & Nikos A. Kouloussis¹

¹Laboratory of Applied Zoology and Parasitology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; evibal@agro.auth.gr

²Laboratory of Remote Sensing and GIS, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

³Laboratory of Agronomy, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Background: Understanding the factors that influence the olive fruit fly's (*Bactrocera oleae*) (Rossi) (Diptera: Tephritidae) ability to resist water deprivation is crucial for pest management and ecology. This study investigated the significant main effects of age, sex, and diet, as well as their complex two-way interactions, on water deprivation resistance.

Methods: Water deprivation resistance (hours until death) was measured across 24 experimental treatment combinations (3 age groups × 2 sexes × 2 diets × 2 mating status = 24). ANOVA indicated significant main effects of age ($F(2, 23) = 121.21$, p -value < 0.001), sex ($F(1, 23) = 204.89$, p -value < 0.001), and diet ($F(1, 23) = 17.88$, p -value < 0.001). A Tukey post hoc analysis was applied to examine statistically significant differences between the treatments.

Results: Significant differences in water deprivation resistance were observed:

- Female Superiority: Virgin females (15 and 30 days old) generally had better water deprivation resistance than their male counterparts, regardless of diet. Similar superiority was noted in mated females on the restricted diet compared with males
- Age-Related Decline: Water deprivation resistance decreased with increasing age across all treatments.
- Diet & Mating Status Effect: 15-day-old mated females on the restricted diet endured longer than those on the full diet.

The highest survival rate beyond the median (90 hours) was 96.67%, observed in 15-day-old virgin females on the full diet and mated females of the same age on the restricted diet. Survival beyond 90 hours was over 50% for all virgin females and all 15-day-old mated individuals.

Conclusions: Sex and age had the greatest effect on water deprivation resistance. Female individuals, especially at younger ages and when virgin, demonstrated superior endurance to water loss. This complex interaction of factors is critical for predicting insect survival in arid or changing environments.

Key words: survival; protein; sugar; olive crops; mating status

Abstract

Food or water deprivation induces distinct changes in the Locomotor Activity of *Bactrocera oleae*

Evangelia I. Balampekou¹, Dimitrios S. Koveos¹, Thomas M. Koutsos², Georgios C. Menexes³, Apostolos Kapranas¹, James R. Carey⁴ & Nikos A. Kouloussis¹

¹Laboratory of Applied Zoology and Parasitology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; evibal@agro.auth.gr

²Laboratory of Remote Sensing and GIS, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

³Laboratory of Agronomy, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

⁴Department of Entomology, University of California, Davis, Davis, CA 95616, USA

Background: The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is a key global pest requiring refined management strategies. Understanding how essential resources, specifically food and water, modulate physiological parameters such as locomotor activity is crucial for optimizing the efficacy of control methods, including baiting and mass-trapping. This study investigated the impact of food and water deprivation on the movement patterns of adult *B. oleae*.

Methods: Locomotor activity was quantified in *B. oleae* adults under controlled laboratory conditions. Experiments were designed to isolate the effects of two distinct stress conditions: food deprivation and water deprivation. A control group with access to both food and water was also maintained. Activity was continuously recorded using an automated monitoring system (Trikinetics) over a defined period of 72 hours, and data recorded were analysed to assess the activity patterns across the treatment groups.

Results: Locomotory patterns exhibited strong temporal dependencies, with significant differences observed between daytime and nighttime periods. Activity was highest in food-deprived adults, followed by water-deprived, and then control groups. Daytime activity showed a temporally restricted peak, with substantially higher locomotion recorded during the late period (15:00 to 20:59) compared to the early period (07:00 to 14:59). Activity was also age-dependent: 5-day-old males and females were significantly more active than 45-day-old counterparts. Finally, 30-day-old females exhibited significantly higher locomotory activity than age-matched males during the daytime.

Conclusions: The locomotor behavior of *B. oleae* is significantly influenced by the temporal phase and stress physiology. The findings provide precise behavioral metrics: a strong stress hierarchy, distinct late-day activity peaks, and notable age- and sex-specific differences. These findings offer actionable insights for enhancing pest control strategies that target stress physiology, senescence, and the aging process. Furthermore, this comprehensive dataset is directly applicable to quality control assessments within Sterile Insect Technique (SIT) programs and forms a vital foundation for future research on *B. oleae* under varied stress conditions.

Key words: pest control, olive groves, Tephritidae, activity patterns

Session 1. Biology, Ecology, Physiology and Behavior

Monitoring of *Drosophila suzukii* (Diptera, Drosophilidae) in Romanian vineyards

Andrei Teodoru*¹, Marioara Puscalau², Ionica Dina³, Teodor Podrumar⁴, Ruxandra Stoica⁵, Bogdan Radu⁵, Daniela Dobromir⁴ & Constantina Chireceanu¹

¹Research and Development Institute for Plant Protection, 08 Ion Ionescu de la Brad, 013813 Bucharest, Romania

²Research and Development Station for Viticulture and Oenology, 61 Ștefan cel Mare, 625300 Odobești, Vrancea County, Romania

³Research and Development Station for Viticulture and Oenology Murfatlar, 02 Calea București, 905100 Constanța County, Romania

⁴Research and Development Station for Viticulture and Oenology Miniș, 06 Iuliu Maniu, 317135 Ghioroc, Arad County, Romania

⁵Research and Development Institute for Viticulture and Oenology, 02 Valea Mantei, 107620 Valea Călugărească, Prahova County, Romania

*E-mail: andrei_theodor44@yahoo.com

Background: *Drosophila suzukii*, the Spotted Wing *Drosophila* (SWD) is an invasive pest of Asian origin that infests a wide range of wild and cultivated fresh and ripening soft fruits including grapes. Grapevine is one of the largest crops in Romania, and infestation of grapes by SWD is to be expected, as the pest is confirmed to be present in berry crops. Our objective was to evaluate the presence and dynamics of SWD adults in vineyards and possible damage to grapes in Romania.

Methods: The study was conducted in 2024 and 2025, from July to December, on red and white wine grape varieties in forty vine plots belonging to six vineyards located in four viticultural regions situated in the west (Miniș-Măderat), east (Odobești, Cotești, Panciu), south (Dealul Mare) and south-eastern (Murfatlar) parts of the country. Traps consisting of 0.5ml transparent plastic bottles baited with a mixture of apple cider vinegar and red wine, replaced at a ten days interval, were used to detect and monitor the adult population of *D. suzukii*.

Results: Our results showed presence of SWD adults in the traps used in all vine plots, from the end of August until December. Most captures were observed in November after the grapes had been harvested in September or October. Proportion of SWD females varied, but in all cases they constituted the majority of individuals captured.

Conclusions: The presence of SWD in Romanian vineyards is confirmed and represents an indication that a risk of grape damage is to be expected, and therefore additional monitoring and assessment studies are needed.

Key words: Spotted Wing *Drosophila*, Monitoring, *Vitis vinifera*, Romania

Effect of yeast hydrolysed proportion in the adult diet to the body weight of males of *Bactrocera dorsalis* and *Bactrocera correcta* (Diptera: Tephritidae)

Ha Thi Kim Lien¹, Nguyen Thi Thanh Hien¹, Rui Pereira²

¹ Entomology Division, Plant Protection Research Institute, Viet Nam

² Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria. Email: kimlientta@gmail.com

Background: Yeast hydrolysed (YH) plays an important role in many activities relating to fruit fly (Diptera: Tephritidae). The change of YH proportion in adult diet can affect the activities/behaviours/characteristic of the sterile fruit fly. In our study, for each adult diet we used a different YH proportion to understand its impact to the body weight of sterile male of *Bactrocera dorsalis* and *Bactrocera correcta*.

Methods: Pupae of *B. dorsalis* and *B. correcta* were radiated at 90 Gy and 80Gy, respectively. 30 males recovered from irradiated pupae at the same weight were released into a screen cage (50cm x 50cm x 50cm in W: L: H) right after emergence. Water and tested diets were provided in a petri dish inside the cage. Males were fed six types of diet since 1 day old. Six tested diets were (T1) sugar (only); (T2) yeast hydrolysed mixed sugar with ratio 1:2; (T3) yeast hydrolysed mixed sugar with ratio 1:4; (T4) yeast hydrolysed mixed sugar with ratio 1:6; (T5) yeast hydrolysed mixed sugar with ratio 1:12; (T6) yeast hydrolysed mixed sugar with ratio 1:3. Un-irradiated females were coupled with above male flies after 9 days of treatment. Then, we measured the weight of mated male collected by micro-balance (OHAUS@ – PA214 - China. Max cap 210gr (reader ability: 0.00001gr). The data were analyzed using Irristat 4.0.

Results: The body weight of mated sterile *B. dorsalis* males ranged from 12.3 - 16.8 mg, of which the lowest (12.3 mg) for males fed on sugar (T1) and the highest (16.8 mg) for males fed on yeast hydrolysed mixed with sugar at ratio of 1:2 (T2). This indicator in other treatments ranged from 13.9 - 16.5 mg, implying a significant difference in the average body weight of males in treatments ($P \leq 0.05$). Sterile males of *B. correcta* that mated in the treatments with protein supplement were weighed to reach an average of 11.4 mg (T5), 11.6 mg (T4), 12.0 mg (T3), 12.2 mg (T2 and T6), respectively. For mated males fed only sugar, the average body weight was 10.2 mg and there were significant differences among treatments ($P \leq 0.05$). Hence, Yeast hydrolysed proportion in the adult diet posed impact on the body weight of sterilized males of *B. correcta* and *B. dorsalis*.

Conclusions: This study as a reference on proportion of YH in adult diet for sterile fruit fly rearing of *B. dorsalis*, *B. correcta* before release.

Keywords: *Bactrocera correcta*, *Bactrocera dorsalis*, yeast hydrolysed, body weight

Session: ~~Biology, Ecology,~~
~~Physiology and Behavior~~

Avoiding competition before it begins: fruit choice mediated by prior interspecific infestation in three fly pests

Valeria A. Viana^{1,2}, María Clara Liendo³, Diego F. Segura^{3,4} & Juan P. Bouvet¹

¹ Estación Experimental Agroindustrial Concordia (INTA), Concordia, Argentina

² CONICET, Argentina

³ Instituto de Genética (INTA) – GV IABIMO (CONICET), Argentina

⁴ Instituto de Investigación en Ciencias Agrarias y Veterinarias, Universidad del Salvador, Argentina

E-mail: viana.valeria@inta.gob.ar

Background: *Anastrepha fraterculus* and *Ceratitis capitata* (Tephritidae), and *Drosophila suzukii* (Drosophilidae) are responsible for significant economic losses in Argentina. These species overlap in their geographic distribution and host fruits, creating scenarios of interspecific competition. One strategy to mitigate such interactions is the use of host-marking pheromones (HMPs), chemical cues that indicate prior fruit occupation. Although their interspecific effects have been demonstrated in *C. capitata* and *A. fraterculus*, it is unknown whether *D. suzukii* can recognize these pheromones/signals. In this study, we evaluated the responses of *A. fraterculus*, *C. capitata*, and *D. suzukii* to fruits previously used by heterospecific females.

Methods: Behavioral assays were conducted in which wild females were offered fruits (plums) previously infested by another species versus non-infested fruits. We recorded latency time, fruit choice, time spent on the chosen fruit, number of ovipositions, and oviposition duration. Five experiments were performed: (E1) *A. fraterculus* exposed to fruits infested by *D. suzukii* (Af vs Ds); (E2) *C. capitata* exposed to fruits infested by *D. suzukii* (Cc vs Ds); (E3) *D. suzukii* exposed to fruits infested by *A. fraterculus* (Ds vs Af); (E4) *D. suzukii* exposed to fruits infested by *C. capitata* (Ds vs Cc); and (E5) *D. suzukii* exposed to fruits infested by conspecifics (Ds vs Ds). In all cases, infested fruits were compared with non-infested fruits.

Results: Significant differences in the number of visits were observed in the Ds vs Cc and Ds vs Af experiments. In both cases, *D. suzukii* visited non-infested fruits more frequently than fruits infested by *C. capitata* or *A. fraterculus*. In the Ds vs Cc experiment, *D. suzukii* spent more time on non-infested fruits than on fruits infested by *C. capitata*. No significant differences were detected in latency time among treatments. In the intraspecific Ds vs Ds treatment, no differences were observed for any variable. The number and duration of ovipositions could not be analysed due to their low frequencies.

Conclusions: These results suggest that *D. suzukii* detects interspecific infestation chemical cues and avoids infested fruits, thereby reducing larval competition. Furthermore, the longer residence time of *D. suzukii* females on non-infested fruits may indicate a preference for these fruits, potentially reflecting recognition once females are on the host. This study provides insights into the role of chemical cues in host selection in these important fruit fly pests.

Key words: Interspecific competition, infested fruit, Drosophilidae, Tephritidae.

Developmental plasticity during the pupal stage modulates adult cold tolerance of the invasive tephritids *Bactrocera dorsalis* and *Bactrocera zonata*?

Vasilis G. Rodovitis^{1,2}, Rui Pereira², Jhonatan S. Aguilar² and Nikos T. Papadopoulos^{1*}

¹ Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Volos, Greece

² Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, Vienna, Austria

Background: Thermal conditions experienced during development can induce phenotypic plasticity that shapes adult stress tolerance in insects, including true fruit flies (Diptera: Tephritidae). Such developmental carry-over effects may be especially important for invasive species of tropical origin that are expanding into temperate regions. Temperature is a primary determinant of developmental rate, adult performance, and overwintering potential; however, relatively few studies have examined how the thermal environment during the pupal stage influences adult cold tolerance. We investigated how temperature during pupae development affects adult cold tolerance in two invasive tephritids: the *Bactrocera dorsalis* and the *Bactrocera zonata*.

Methods: Pupae of both species were reared under three constant temperatures (15, 20, and 25 °C). The effect of temperature during development on Critical Thermal Minimum (CTmin) and Chill Coma Recovery Time (CCRT) of the emerging adults was recorded. Fruit fly species and sex were considered in data analysis.

Results: CTmin of adults emerging from pupae reared at 15 °C was significantly lower than that of adults from pupae reared at 25 °C in *B. dorsalis*, whereas developmental temperature had no effect on adult CTmin in *B. zonata*. In both species adults obtained from pupae developed at 15 °C recovered from chill coma significantly faster than those from 20 and 25 °C. *Bactrocera dorsalis* displayed stronger temperature-dependent variation and slower overall recovery than *B. zonata*, but both species converged in recovery performance at 15 °C. This suggests that cooler temperatures during pupae development enhance cold tolerance primarily through improved recovery time. The observed interspecific differences reflect distinct cold tolerance patterns between the two species, *B. dorsalis* from tropical, rather no seasonal environments exhibiting higher plasticity, and *B. zonata* from subtropical regions showing more canalized cold responses.

Conclusions: These findings highlight developmental temperature as a key aspect of adult cold performance and invasion potential in tephritids. Incorporating developmental plasticity into bioclimatic and pest risk models will improve predictions of invasion success, establishment probability, and range expansion by driving overwintering success of such species under climate change.

Key words: Developmental acclimation; phenotypic plasticity; chill coma recovery; critical thermal minimum; invasion biology; fruitflies

Long term population seasonal variations of major fruit fly species and the possibility of displacement of indigenous species by *Bactrocera dorsalis*

Vincent Umeh, Vivian Umeh and Olanrewaju Shittu

National Horticultural Research Institute, P.M.B. 5432, Idi-Ishin, Jericho Reservation Area, Ibadan, Nigeria. E-mail: vumeha@yahoo.com

Background: Fruit fly attacks on mango constitute an important problem to the growth of the horticultural industry in Western Africa. They destroy 30 to 80% of mango fruits if no control measure is applied. With the advent of *Bactrocera dorsalis* in West Africa and its invasive nature on mango, an investigation was initiated to determine its status *vis a vis* over other existing species during a 7 year period (2018–2025). The study has the objective of determining the seasonal variation in the populations of important mango-attacking species and their population dynamics. The issue of displacement of the indigenous species by the invasive *Bactrocera dorsalis* was also assessed.

Methods: Faced with the pressure of fruit flies on horticultural productions in Nigeria, data of surveys on mango were conducted between 2018 - 2025 to address the above objectives. Fruit fly population data were obtained from three selected commercial mango orchards (of not less than 8 ha) in three states of Nigeria, namely Benue, Nasarawa and Plateau states which are located in the Guinea savanna agro-ecological zone. Tephri traps separately baited with 4 different types of pheromone that are attracted to the major fruit fly species present in Nigeria were deployed. Three orchards were used as replicates. The number of fruit flies captured by the traps were recorded at weekly intervals. The temperature, relative humidity and rainfall data of the period covered by the surveys were also recorded.

Results: The result showed that *B. dorsalis* population dominated trap catpures (98%). Other species of importance observed included *Ceratitis cosyra* (Walker), *Zeugodacus cucurbitae* (Coquillett), *C. brevi* (Guérin-Mèneville), *C. capitata* (Wiedemann) and *Dacus bivittatus* (Bigot). Generally, the weather condition (temperature, relative humidity and rainfall) seem to play a major role in the population dynamics of fruit flies of mango. The population studies show that *B. dorsalis* is most prevalent in the rainy seasons (mean number of 35 - 56/trap/day in June) than in the dry season (mean number of 1.3 - 9.2/trap/day in February); while the mango fruit fly, *C. cosyra*, abound mostly in the dry season months (mean number of 4 - 17/trap/day in February compared to 0 - 6/trap/day in June). Displacement of the indigenous species on mango by the invasive *B. dorsalis* and the resurgence of the indigenous species with time is discussed. Other factors that are likely to affect the observed fruit fly species' populations is also discussed.

Conclusions: The study shows that weather factors play a major role in the population dynamics of mango-attacking fruit flies. Dominance and displacement of indigenous species may occur with the presence of invasive species such as *B. dorsalis*

Key words: *Bactrocera dorsalis*, *Ceratitis cosyra*, Oriental fruit fly, Population dynamics

**Males regurgitate triggers sexual attraction and reproductive maturation in
Anastrepha fraterculus (Diptera: Tephritidae)**

**Micaela S. Garbalena^{1,2}, Ana L. Nussenbaum^{1,2,3}, Guillermo E. Bachmann^{1,2},
Teresa M. Vera^{2,4,5} & Diego F. Segura^{1,2,3}**

¹ Instituto de Genética, CICVyA, INTA, Argentina. mail: [garbalena.micaela @inta.gob.ar](mailto:garbalena.micaela@inta.gob.ar)

² CONICET, Argentina

³ Instituto de Investigación en Ciencias Agrarias y Veterinarias, USAL, Argentina

⁴ Facultad de Agronomía y Zootecnia, UNT, Argentina

⁵ Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, IAEA, Austria

Background: The Diptera crop is a specialized digestive organ that enables flies to store, concentrate, and regulate liquid food intake through controlled movement of its contents, including the regurgitation of crop fluids, a behavior that allows flies to externally deposit, re-ingest, or share regurgitate droplets with conspecific individuals. Beyond its primary nutritional role, this behavior has been shown to contribute to water balance and other very diverse roles, such as thermoregulation, detoxification, and microbial transmission. In tephritid fruit flies, regurgitation has gained additional relevance in sexual contexts. Particularly for *Anastrepha suspensa*, evidence indicates that males use regurgitated droplets during courtship by depositing pheromone-laden droplets containing pheromonal compounds in the environment, thereby enhancing mate attraction. To date, little is known about the regurgitation behavior of *Anastrepha fraterculus*, the South American fruit fly.

Methods: Experiments were conducted under controlled laboratory conditions combining chemical, behavioral, and physiological approaches to evaluate the content of regurgitated crop fluid and its role in sexual interactions in *A. fraterculus*. Regurgitates from immature and mature flies were collected using a graduate capillary and extracted with hexane. Chemical characterization was conducted using gas chromatography-mass spectroscopy. The attractiveness of males regurgitate to virgin females was tested using a Y-tube olfactometer. The effects of regurgitate consumption on female mating propensity and ovarian development were assessed through mating assays and morphometric analyses.

Results: Only mature male regurgitate contained epianastrephin and anastrephin, in proportions like those reported for the lekking pheromone. No additional pheromonal compounds were found. The regurgitate from mature males was significantly more attractive to adults—particularly females—in olfactometer assays compared to immature male samples (Exact binomial test, $p < 0.01$). Moreover, the consumption of male regurgitate increased the probability of copulation in females ($\chi^2 = 9.07$, d.f. = 1, $p < 0.01$), and it also promoted greater ovarian development ($\chi^2 = 8.85$, d.f. = 1, $p < 0.01$).

Conclusions: Our results extend previous findings in *A. suspensa* to *A. fraterculus*, indicating that male-derived regurgitate contains pheromonal compounds that positively influence attraction and female reproductive investment. These findings may enrich current knowledge on the roles of regurgitation and provide new potential information for the development of strategies to control these pest species.

Key words: Crop, chemical ecology, drop, lekking, South American fruit fly

Comparative transcriptomic analyses provide insights into the ecological driving force behind odorant receptor evolution in Tephritidae

Emma Persyn^{1,2}, Pierre-François Duyck^{3,4}, Marie-Christine François², Christian Mille³, Vincent Jacob¹, **Emmanuelle Jacquin-Joly**²

¹CIRAD, Université de la Réunion, UMR PVBMT, 7 ch. de de l'IRAT, F-97410 Saint-Pierre, La Réunion, France

²INRAE, Sorbonne Université, CNRS, IRD, UPEC, Université Paris Cité, Institute of Ecology & Environmental Sciences of Paris, F-78026 Versailles cedex, France

³IAC, Institut Agronomique néo-Calédonien, Equipe ARBOREAL, Laboratoire d'Entomologie Appliquée, Station de Recherches Fruitières de Pocquereux, F-98880 La Foa, New Caledonia

⁴CIRAD, UMR PVBMT, F-98488 Nouméa, New Caledonia

E-mail: emmanuelle.joly@inrae.fr

Background: The insect olfactory system has evolved to guide species toward specific mating partners, food sources, and oviposition sites. However, how species-specific repertoires of odorant receptors (ORs)—which mediate the detection of volatile cues—have been shaped by ecological pressures remains poorly understood. Fruit flies of the Tephritidae family (Diptera) exhibit highly diverse host preferences due to multiple host switches throughout their evolutionary history, making them ideal models to explore this question.

Methods: In this study, we used RNA sequencing to manually identify the OR repertoires of thirteen Tephritidae species with varying host ranges: *Bactrocera curvipennis*, *Bactrocera dorsalis*, *Bactrocera psidii*, *Bactrocera tryoni*, *Bactrocera umbrosa*, *Bactrocera zonata*, *Ceratitis capitata*, *Ceratitis catoirii*, *Ceratitis quilicii*, *Dacus ciliatus*, *Dacus demmerezii*, *Neoceratitis cyanescens*, and *Zeugodacus cucurbitae*, and conducted differential expression analyses. We next searched for significant correlations among OR characteristics, species host range, and phylogenetic position.

Results: We annotated 60-80 OR transcripts per species, including the obligatory coreceptor *Orco*, and identified a total of 698 new ORs. Differential expression analyses revealed some organ- and sex-biased OR expression patterns. Importantly, after correcting for phylogenetic distance, we found significant correlations between certain OR repertoire characteristics and host range. Specifically, both the sequence and relative expression levels of several ORs were more conserved in polyphagous than in oligophagous species, and certain ORs were uniquely present in polyphagous species.

Conclusions: This work brings new resources for the fruit fly community and provide molecular insights into the ecological forces shaping OR evolution in Tephritidae fruit flies.

Key-words: Odorant receptors, RNAseq, Phylogeny, Differential expression, Host range

Gene Flow and Abundance of a Tropical Fruit Fly in a Horticultural Landscape Mosaic in Eastern Australia Is Limited by Cleared Grazing Land and Area-Wide Management

James L Ryan^{1,2}, Anthony R. Clarke¹, Alexander M. Piper³, Susan Fuller¹ & Peter J. Prentis^{1,2}

¹School of Biology and Environmental Science, Queensland University of Technology, Brisbane, Queensland, Australia

²Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, Queensland, Australia

³Agriculture Victoria Research, AgriBio Centre, Bundoora, Victoria, Australia

Mail: j56.ryan@hdr.qut.edu.au

Background: Landscape ecology and genetics provide important analytical frameworks for investigating the effect of environmental features on ecological processes. Few empirical studies, however, have simultaneously tested how landscape characteristics influence spatial patterns of gene flow and abundance of pest insects in heterogeneous environments. To address this, we undertook a combined landscape-ecology/landscape-genetic study of the tephritid fruit fly, *Bactrocera tryoni*, in the Wide Bay-Burnett region of Southeast Queensland, Australia. This region contains a diverse array of landscapes including two areas of intensive horticulture production; one implementing area-wide management practices. We also collected a representative sample of local Dacini species which can be used to model assembly patterns across different ecosystem types.

Methods: We collected Dacini fruit fly samples, including *B. tryoni*, from 26 sites in 2021 during the months of April, August, October, December and the following year during February and April. We used high-density DArTseq SNP genotyping on *B. tryoni* samples collected during the 2021 April, August and December sampling periods. We then modelled the contemporary landscape characteristics and management factors influencing gene flow and abundance of this pest species. We also investigated differences in Dacini assemblages using several diversity measures and dissimilarity modelling.

Results: Genome-wide SNP analysis estimated infinite effective population sizes at all sites and detected limited genetic structure across the landscape. However, *B. tryoni* abundance varied significantly among habitats, with cleared pasture negatively associated with population abundance and acting as a barrier to gene flow. Additionally, highways in composite with cleared pasture exhibited a very strong barrier effect. *B. tryoni* abundance was reduced in the horticultural region with area-wide management implemented. We also found that assemblage composition in both abundance and species richness was significantly different between rainforest and all other ecosystem types.

Conclusions: Our analysis highlights the benefits of collecting simultaneous genetic and ecological datasets for informing and evaluating area-wide management programmes for insect pests and highlight considerations in the spatial analysis of SNP data when effective population sizes are extremely large.

Key words: *Bactrocera tryoni*, effective population size, landscape ecology, landscape genetics, linear mixed modelling, Queensland fruit fly, SNPs

**Spatio-temporal genetic variation in a local population
provides insights into off-season dynamics of a crop pest**

Cécile Caumette, Karine Berthier, Laure Benoit, Massamba Diakhaté, Anais
Chailleux, Thierry Brévault, Raphaël Leblois, **Marie-Pierre Chapuis**

CBGP, CIRAD, INRAE, IRD, Montpellier SupAgro, University of Montpellier, Montpellier,
France.

E-mail: marie-pierre.chapuis@cirad.fr

Background: Quantifying demographic parameters such as population size and dispersal is essential to understanding the seasonal persistence and re-infestation dynamics of pest species in fluctuating agricultural landscapes, and to informing sustainable management strategies. While genetic approaches can provide valuable indirect insights, their application to pest species particularly complex due to inherently large parameter values and demographic disequilibrium.

Methods: In this study, we addressed this challenge through a case study of *B. dorsalis*, a major pest of mango crops showing seasonal population eruptions and collapses. We used longitudinal demographic and genetic data collected from a fine-scale area in the Senegalese sub-Sahel, which sustains populations during the dry and fruitless season. We quantified temporal changes in genetic variation over two seasonal demographic cycles in the refuge population and estimated population size and dispersal under isolation-by-distance, while validating methods through simulations.

Results: Our study provides indirect evidence of immigration at the onset of population growth and genetic drift during the off-season. While high population densities limited reliable parameter estimation during the growth phase, we estimated effective population sizes ranging from several hundred to several thousand individuals during the off-season. Using these estimates to inform local density, we derived an average dispersal distance of approximately one kilometer from neighborhood size estimates. However, those quantities estimated during low-density phases are expected to be underestimated due to downward bias and genetic time lag.

Conclusions: In conclusion, despite inherent methodological limitations, our study, based on empirical and simulated data, demonstrates the potential and limits of the genetic approach in the challenging context of rapid population cycles, providing useful insights for future work.

Key words: *Bactrocera dorsalis*, Oriental fruit fly, seasonal dynamics, population genetics, isolation by distance, effective population size, demographic inference, simulations

Overwintering Capacity of the Medfly in the Dalmatia Region of Croatia

Mario Bjeliš¹, Ivan Tavra¹, Luka Popović², Frane Strikić¹, Cleopatra A. Moraiti³,
Vasilis G. Rodovitis and Nikos Papadopoulos³

¹ Agromediterranean Faculty, University of Split, Zrinsko-Frankopanska 38, 21000 Split, Croatia, mbjelis@unis.hr

² Center for Plant Protection, Croatian Food Agency, Tisno bb, 20355 Opuzen, Croatia

³ Laboratory of Entomology and Agricultural Zoology, School of Agricultural Sciences, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Fytokou St., 38446 Volos, Magnesia, Greece

Background: The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is considered one of the most important phytophagous pests worldwide. In Croatia it severely affects the fresh fruit production in Dalmatia, and a national suppression action plan is in force to alleviate farmers' burden. The aim of the current paper was to comprehensively study the overwintering dynamics of medfly adults in the Dalmatia and to investigate whether adults can overwinter in different conditions, with an ultimate goal to support the establishment of a sound off-season control strategy for this pest.

Methods: Overwintering trials took place in Split (Dalmatia region, Croatia) using the local *C. capitata* population. Cages with different developmental stages (adults, pupae) or infested fruits were periodically transferred in three overwintering sites (open field, semi field and urban conditions) from October to December (two to three establishment dates during 2019-2020 and 2020-2021 season).

Results: Our results provide strong evidence that, besides larvae within fruit, adults can survive at significant proportions in open field and urban conditions. Overwintering site and establishment date were both significant predictors of the overwintering success of adult developmental duration, female fecundity and generally the overwintering ability.

Conclusions: Overwintering site and establishment date were both significant predictors of the overwintering success of adults, and they affect pupal developmental duration, adult emergence rates from overwintering pupae, female fecundity, and generally the overwintering ability of the pupae. Our results suggest that the current national strategy for *C. capitata* suppression, which is based on the assumptions of larvae overwintering need to be adjusted by development and implementation of the off-season strategy.

Key words: field survival; adult survival; pupae developmental duration; off season; longevity; lifespan; fecundity; overwintering refugia

Crop regurgitation mitigates the lethal effects of spinosad in *Anastrepha fraterculus* (Diptera: Tephritidae)

Micaela S. Garbalena^{1,2}, Guillermo E. Bachmann^{1,2}, Francisco Diaz Fleischer³; Teresa M. Vera^{2,4,5} & Diego F. Segura^{1,2,6}

¹ Instituto de Genética, CICVyA, INTA, Argentina. Email: garbalena.micaela@inta.gob.ar

² CONICET, Argentina

³ INBIOTECA, Universidad Veracruzana, México

⁴ Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, IAEA, Austria

⁵ Facultad de Agronomía y Zootecnia, UNT, Argentina

⁶ Instituto de Investigación en Ciencias Agrarias y Veterinarias, USAL, Argentina

Background: The insect crop may function as a first barrier against toxic dietary compounds. Regurgitation provides a rapid, behavioral mechanism to reduce toxin load, allowing the insect to expel contaminated crop contents prior to intestinal absorption. Here, we evaluated the impact of regurgitation after the ingestion of Spinosad in *Anastrepha fraterculus*.

Methods: Two assays were conducted to address the following questions: (1) Is regurgitation behavior modified following ingestion of a toxic substance? and (2) Would disabling regurgitation of the ingested toxin reduce flies' life expectancy? To answer (1), 15 flies were placed individually in acrylic boxes and provided with a 20% sucrose solution supplemented with Spinosad (4.8 ppm), a highly toxic concentration to *A. fraterculus*. The control group received the same diet without Spinosad. Diets were supplemented with a food-grade dye to improve detection of regurgitated droplets, and a piece of filter paper was placed inside each box to allow collection and visualization of the regurgitate. Latency to regurgitate after the first ingestion was recorded, as well as the number of regurgitated droplets at 1, 6, and 24 h post-feeding. To answer (2), flies were offered the Spinosad-containing diet and then assigned to two treatments: proboscis occluded with paraffin (PO+S+) to prevent crop emptying, and individuals that freely regurgitated (PO-S+). An additional control group (PO+S-) received a sucrose diet without Spinosad but had their proboscis occluded. Mortality was recorded individually every day, over a 72 h period (22 flies per treatment).

Results: Flies exposed to Spinosad showed a significantly slower initial regurgitation response compared to control flies ($F = 8.05$, $p = 0.008$). However, Spinosad-treated flies regurgitated a greater number of droplets. The mean number of droplets was 4.1 ($p = 0.0066$), 4.0 ($p = 0.0337$), and 6.8 ($p < 0.0001$) times higher than in the control group at 1, 6, and 24 h post-feeding, respectively. In the second experiment, group PO+S+ exhibited earlier mortality and a higher risk of death compared to PO+S- group ($HR = 9.68$, $p < 0.001$). In contrast, groups PO-S+ and PO+S- maintained a higher proportion of surviving flies throughout the observation period.

Conclusions: Although insect responses to toxic compounds in insects have traditionally been attributed to enzymatic pathways, these findings highlight that behavioral mechanisms such as regurgitation may play a complementary role by limiting toxin absorption at early post-ingestive stages, at least in *A. fraterculus*.

Key words: emesis, drop, insecticide, South American fruit fly

Thermal requirements and phenology modelling of *Drosophila suzukii*: implications for pest risk under changing climates

Shepard Ndlela, Esther Awuori, Abdelmutalab G A Azrag, Farid Ibrahim Maholidy & Samira A. Mohamed

International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya

Background: *Drosophila suzukii* (spotted-wing drosophila) is an invasive pest responsible for substantial economic losses in fruit crops worldwide. Following its recent invasion into Africa, the species is rapidly spreading, raising concerns about its establishment and impact under current and future climate conditions. Understanding its temperature-dependent biology is critical for predicting population dynamics and invasion risk.

Methods: Temperature-dependent life table parameters, including developmental time, survival, fecundity, and adult longevity, were quantified for an African *D. suzukii* population collected in Kenya. Experiments were conducted under constant temperatures ranging from 10 to 30 °C, as well as under fluctuating temperature regimes. Linear and nonlinear temperature-response models were developed using ILCYM software to characterize thermal effects on life history traits. Phenology and population growth were simulated, and global spatial risk indices, Establishment Risk Index (ERI), Generation Index (GI), and Activity Index (AI), were projected under current and future climate scenarios (2061-2080).

Results: Developmental thresholds were estimated at 6.87 °C for the egg–larva stage and 9.13 °C for the pupal stage. Optimal development and reproduction occurred between 20 and 27 °C, while mortality increased at thermal extremes, with minimum mortality near 19.9 °C. The highest intrinsic rate of natural increase ($r_m = 0.276 \text{ day}^{-1}$) and finite rate of increase ($\lambda = 1.317$) were observed at 27 °C. Population growth declined sharply above 30 °C, indicating a narrow thermal window for population viability. Spatial projections revealed potential poleward and elevational shifts in suitable habitats, including expansion into northern Europe, southern Canada, and African highlands, alongside contraction in overheated lowland regions.

Conclusions: The study highlights the strong influence of temperature on *D. suzukii* population dynamics and identifies clear thermal limits that may constrain or shift its distribution under climate warming. Region-specific, temperature-driven phenology models are essential for forecasting invasion risk and informing climate-smart pest management strategies. Integrating empirical life table data with mechanistic modelling provides a robust framework for optimizing surveillance and control of *D. suzukii* in Africa and globally under changing climatic conditions.

Key words: Spotted-wing drosophila, ILCYM, invasive pest, temperature-dependent life table, population dynamics; berries

Life-stage morphometric, durations and larval density of solanum fruit fly infesting Akabare chili in Nepal

Sudeep Kumar Upadhyay¹, Kashinath Chiluwal¹, Ajaya Shree Ratna Bajracharya¹, Rakshya Devkota² and Yogyata Neupane³

¹ National Entomology Research Centre, Khumal Complex, Lalitpur, Nepal, ² Agriculture and Forestry University, Rampur, Chitwan, Nepal, ³ Himalayan College of Agricultural Sciences and Technology, Kirtipur, Kathmandu, Nepal
E-mail: — sudeepddl@gmail.com

Background: Recent invasion of solanum fruit fly, *Bactrocera latifrons* (Hendel) (Diptera: Tephritidae) is creating havoc among Akabare chili (*Capsicum annuum*) farmers of Nepal. Despite its increasing economic importance, information on its biology, morphometry, and larval infestation intensity under Nepalese conditions remains limited. Understanding larval development and density within host fruits is essential for accurate pest identification, monitoring, and management strategies.

Methods: Laboratory studies were conducted for the morphometric-based identification; life-stage durations and maggot density in infested fruits were evaluated thereby to facilitate its proper monitoring and management. Field-collected infested chili fruits were sampled from three different commercial farming areas of Nepal to assess larval density. The data were checked for normality and evaluated using one-way ANOVA followed by a Tukey's HSD for mean separation at $P = 0.05$.

Results: The body length differed significantly ($P < 0.001$) among the life stages of the fly, the highest (9.32 ± 0.18 mm) and the lowest (2.0 ± 0.06 mm) for the third and the first instar maggots, respectively. Body width also differed by life-stages ($P < 0.001$), the female adults being the widest (6.87 ± 0.02 mm) and the tiniest being the first instar maggots (0.4 ± 0.02 mm). In terms of weight, the third instar maggots were the heaviest (4.12 ± 0.11 g) and the first instar being the lightest (0.37 ± 0.01 g) thereby differing statistically ($P < 0.001$). The head capsule diameter was widest for the third instar larvae (0.71 ± 0.01 mm) making it significantly the largest ($P < 0.001$). The female adults (9.00 ± 0.71 days) and pupae (9.00 ± 0.00 days) survived equally the longest compared to other life stages ($P < 0.001$). The maggots per 100 g of infested fruits were not significantly different ($P = 0.78$) for those collected from the tested three locations suggesting for the similar infestation level across the locations.

Conclusions: The study provides basic biological and morphometric data for *B. latifrons* in Nepal and information is about the pest's potential to damage. These findings contribute valuable information for accurate identification, population assessment, and the development of effective management strategies against *B. latifrons* in chilli-growing regions of Nepal.

Key words: *Bactrocera latifrons*, chilli, larval biology, larval density, morphometry,

Seasonal abundance of melon fly *Zeugodacus cucurbitae* (Coquillett) in relation to abiotic factors and host fruits in Western Burkina Faso

Issaka Zida, Alizèta Sawadogo, Karim Nébié & Souleymane Nacro

Centre National de la Recherche Scientifique et Technologique/Institut de l'Environnement et de Recherches Agricoles/Laboratoire Central d'Horticulture, Station de Farako-Ba, Bobo-Dioulasso, Burkina Faso, E-mail: ishakazida@gmail.com

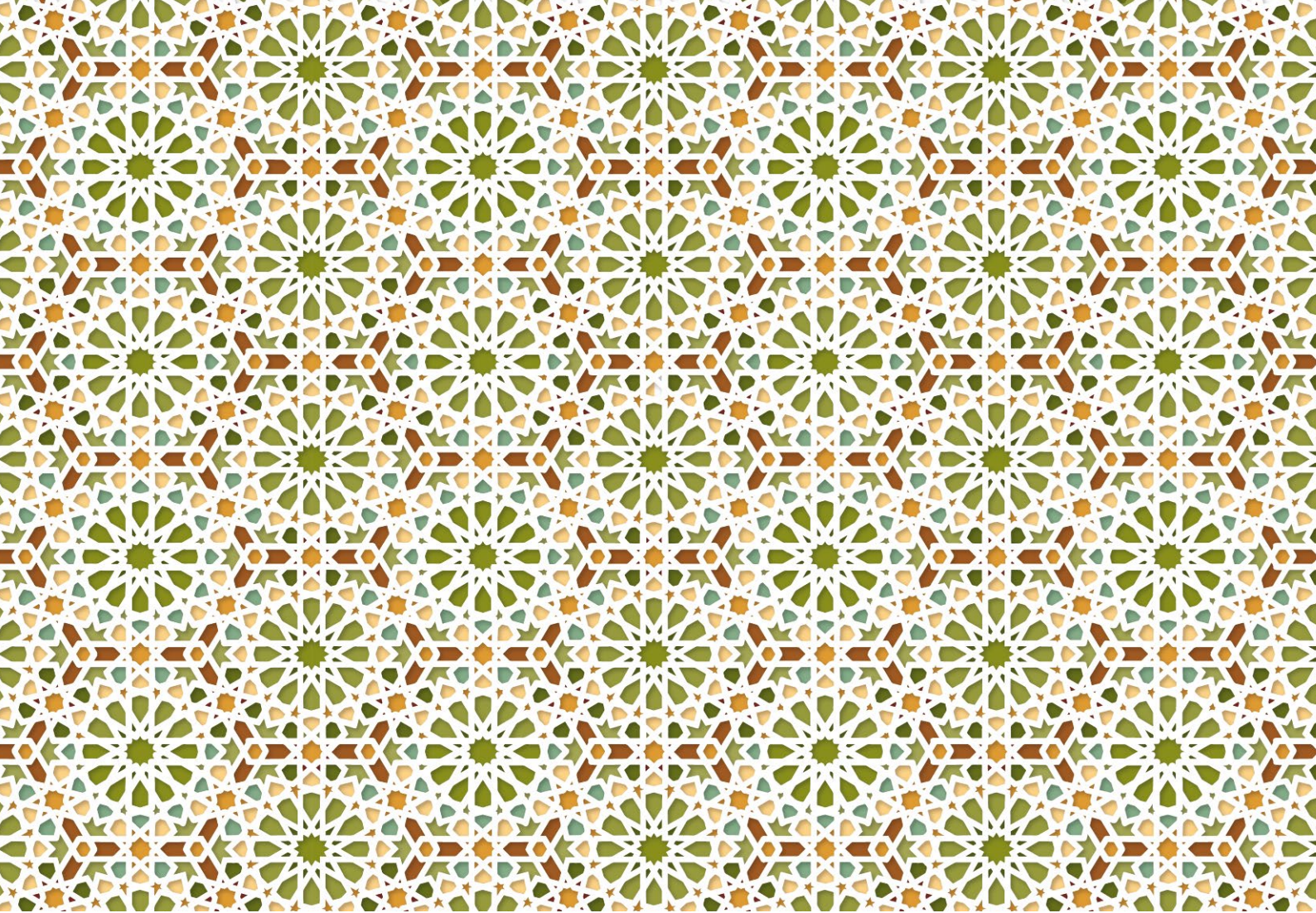
Background: In Western Burkina Faso, fruits and vegetable industry is facing serious threats from fruit fly pests. These have been exacerbated with the invasion of exotic and invasive fruit fly pests including *Bactrocera dorsalis* and *Zeugodacus cucurbitae*. While recent studies investigated the bioecology and development of *B. dorsalis* control approaches in West Africa, less is known about *Z. cucurbitae* and factors that influence its population dynamics in plant formations in south Sudanese zone of Burkina Faso. This study fills a gap in information on abiotic factors and host plant suitable for *Z. cucurbitae* fluctuations in Western Burkina Faso.

Methods: Seasonal fluctuations of *Z. cucurbitae* was investigated through a trapping system in both vegetable gardens and areas where vegetable crops were not grown. The male lure, Cuelure associated with a killing agent, dichlorvos (DDVP) were used in tephri trap types for monitoring *Z. cucurbitae* populations in different vegetation types over (2022-2024) in six localities. Sampling, collection and fruit holding were performed in order to identify fruit species that act as hosts for *Z. cucurbitae* females.

Results: *Zeugodacus cucurbitae* represented 59,39% and 52,93% of adult flies captured in traps placed in market gardens and biotopes where vegetable crops were not grown, respectively. The flies per trap per day (FTD) of *Z. cucurbitae* varied significantly during the experimentation depending on the survey period. This species was absent in the different vegetation types during the dry and hot seasons of the year especially in natural areas. *Z. cucurbitae* population peaked in October irrespective to the year and vegetation types. Its population density was higher in cultivated areas compared to the natural ones. This exotic fly dominated native *Dacus* species in trap catches. Five fruit species of Cucurbitaceous family were infested with *Z. cucurbitae* including one wild fruit species with various damage indices (name them?).

Conclusion: In our study area, *Z. cucurbitae* population dynamics were influenced by temperature, relative humidity and the availability of host plants. This study demonstrated that *Z. cucurbitae* dominated native *Dacus* species in trap catches. However, despite its predominance in trap catches, the native *Dacus* species dominated *Z. cucurbitae* in co-infested vegetable crops. As a result, the probable coexistence of the exotic species and the residents can be hypothesized in the study zone.

Keywords: *Zeugodacus cucurbitae*, dynamic, host plants, fluctuation, Burkina Faso



POSTERS

Taxonomy and Morphology

FIRST DETECTION, IDENTIFICATION AND ERADICATION OF ORIENTAL FRUIT FLY, *BACTROCERA DORSALIS* (HENDEL) (DIPTERA: TEPHRITIDAE), IN ZF MGCWU AND PIXLEY KA SEME DISTRICT MUNICIPALITIES, IN NORTHERN CAPE PROVINCE, SOUTH AFRICA

Emily N. Munganga¹, Onkabetse Lentswe², Tertia Grove³

¹Department of Agriculture, Plant Health, Pretoria, Gauteng, South Africa

²Department of Agriculture, Inspection Services, Upington, Northern Cape, South Africa

³ARC-TSC Corner Bosch Street and Kanyamazane Road R2296, Mbombela | 1200, Mpumalanga, South Africa; E-mail: EmilyMu@nda.gov.za

Background: Northern Cape Province consists of six district municipalities. The ZF Mgcawu and Pixley Ka Seme District Municipalities produce table grapes and citrus as some of key crops, generating significant income through commercial farming. *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), Oriental Fruit Fly (OFF), a regulated pest in South Africa, according to Control Measures, R. 110, in terms of the Agricultural Pests Act, 1983 (Act No. 36 of 1983), was first introduced in South Africa in 2010. Since 2013, the status of OFF in South Africa has changed from having areas where the pest was transient, actionable and under eradication, with other areas free from this pest and others where the pest is present. OFF was first detected in Northern Cape, between February and April, in 2018, in ZF Mgcawu District Municipality, and later observed in Pixley Ka Seme District Municipality, between February and May, in 2019.

Method: Several suspect specimens were collected from several ME-baited traps, from commercial table grapes and citrus orchards. The pre-identification was conducted morphologically, using ‘A set of multi-entry identification keys to African frugivorous flies (Diptera:Tephritidae)’. Molecular technique, using Polymerase Chain Reaction (PCR), confirmed the species identification. Phytosanitary actions were implemented, with joint collaboration between the Department of Agriculture (DOA), the growers, the public and private sectors, as eradication efforts to control the pest. This included the use of deployment of male annihilation technique (MAT) and weekly application of protein bait spays, as well as the prohibition of movement of host material and sanitation.

Results: Both identification methods conducted, resulted in male specimens of OFF. No further detections were experienced after the last specimen was detected. The areas were monitored for more than 12 weeks, after more than three OFF generations. areas, i.e, eradicated.

Conclusion: Coupling morphological and molecular techniques ensures proper and efficient identification processes. Strict quarantine measures implemented, led to successful declaration of absence of the pest in ZF Mgcawu and Pixley Ka Seme District Municipalities, by early 2018 and late 2019, respectively. All district Municipalities, in Northern Cape Province, are currently free from OFF, and the fruit fly surveillance continues in the area.

Key words: *Bactrocera dorsalis*, detection, eradication, pre-identification, morphological, control
Session 2: Taxonomy and Morphology

Henriette Rasolofoarivao^{1,2*}, Marc De Meyer³, Massimiliano Virgilio³, Lala Harivelo Ravaomanarivo² and **Hélène Delatte**^{4*}

Background: Tephritid fruit flies are among the most important pests of horticultural crops in the Indian Ocean region. Madagascar, a tropical continental island and biodiversity hotspot, hosts a particularly rich tephritid fauna, with 75 described species, including 32 frugivorous species whose larvae attack fruits and vegetables of economic importance. Five species are considered major agricultural pests, three of which are endemic, while *Bactrocera dorsalis*, detected in 2010, is the most recent and damaging invasive species. Despite the long history of tephritids on the island, knowledge of their current population dynamics and community composition in natural habitats remains limited. In particular, no large-scale surveys of tephritid populations have been conducted in those natural habitats for more than a decade. This study aims to characterize the population dynamics and species diversity of Tephritidae in protected natural areas of Madagascar.

Methods: Fruit fly populations were monitored in seven natural parks (north: Montagne d'Ambre and Ankarana, northwest: Ankarafantsika, east: Ambohidray, southeast: Ranomafana and Andohahela, southwest: Kirindy) across Madagascar using a standardized trapping protocol and fruit collecting. Tephri-traps were deployed and baited with species-specific and general attractants, including methyl eugenol, cuelure, trimedlure, terpinyl acetate, biolure, egolure, and zingerone. Each trap was equipped with a DDVP killing strip. Traps were checked every month over a period of six to eight months, covering key seasonal variations. Lures and killing strips were replaced monthly to maintain trapping efficiency. Captured specimens were collected, identified to species level, and used to assess species composition, relative abundance, and temporal population dynamics within and among natural parks.

Results: Trapping revealed a diverse assemblage of tephritid species across all surveyed parks, reflecting the high biodiversity of Madagascar's natural ecosystems. Both endemic and exotic species were recorded, with marked differences in species composition and abundance among sites. Population dynamics varied seasonally, with distinct peaks associated with climatic conditions and resource availability.

Conclusions: This study provides the first multi-site, multi-lure assessment of tephritid population dynamics in natural parks of Madagascar. Our results highlight the coexistence of endemic and invasive fruit fly species within protected ecosystems and underline the role of natural habitats in shaping tephritid communities. The detection of *B. dorsalis* was found close to the border of natural parks, where non-native commercial crops were mixed with natural forest, which raises important questions regarding its ecological impact with native species and natural enemies.

Key words: fruit flies, forest, surveys, trap

THE THISTLE FLIES OF THE GENUS *CHAETOSTOMELLA* (DIPTERA: TEPHRITIDAE: TERELLIINI) FROM MOROCCO, WITH A REFERENCE TO EUROPEAN AND ASIAN FAUNA

Younes El Harym^{1*}, Andrii M. Troshyn², Robert Schreiber³ & Valery A. Korneyev^{2, 3}

¹LESCB URL-CNRST N°18, FS, Abdelmalek Essaadi University, Tetouan, Morocco
elharym.younes@gmail.com; <https://orcid.org/0000-0002-6852-6602>

²I.I.Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Bogdan Khmelnytsky St. 15, 01054 Kyiv, Ukraine

andrey.troshin2275@gmail.com; <https://orcid.org/0000-0002-6917-3167>
valery.korneyev@gmail.com; <https://orcid.org/0000-0001-9631-1038>

³Museum für Naturkunde, Invalidenstr. 43, 10115 Berlin, Germany

*Corresponding author

Abstract :

The genus *Chaetostomella* Hendel, 1927 (Diptera: Tephritidae) in the fauna of Morocco is reviewed based on integrative morphological and molecular analyses. Phylogenetic reconstruction using a concatenated dataset of four genes (COI, 16S, 28S, AATS) strongly supports the monophyly of the genus and resolves *Chaetostomella steropea* as the basalmost lineage, sister to the recently radiated *Chaetostomella cylindrica* species complex. Two previously unknown species, *Chaetostomella* sp. n. 1 (reared from *Cynara scolymus* and *Carthamus pectinatus*), and *Chaetostomella* sp. n. 2 (reared from *Mantisalca salmantica* and *Ptilostemon rhiphaeus*) are discovered. Despite showing moderately low genetic divergence from *C. cylindrica*, which indicates their evolutionary youth, both new species exhibit stable diagnostic morphological characters and geographic isolation. A previously poorly known *Chaetostomella steropea* (Rondani, 1870) is redescribed based on specimens reared from *Chamaeleon gummifer*, representing its first record for North Africa. In addition, *Chaetostomella cylindrica* is shown to be a large complex of genetically close host races and geographic forms occurring throughout the Western Palaearctic. Further detailed studies are required to understand their host-plant specialization and the evolutionary trajectories of these "thistle flies."

Key words: new species, host plant, North Africa, Rif region, phylogeny, DNA barcoding, Asteraceae, Cardueae, *Carthamus*, *Chamaeleon*, *Cynara*, *Mantisalca*, *Ptilostemon*.

Potential to use wing shape variation as a novel approach to discriminate *Zeugodacus cucurbitae*, *Z. caudatus*, and *Z. tau* fruit flies (Tephritidae)

Udekumari P. G. N. C., Gamage L. M. P., Wijekoon W.M.C.D*., Wegiriya H.C.E.

Department of Zoology, University of Ruhuna

* chandanadammika1984@gmail.com

ABSTRACT

Background: *Zeugodacus* fruit flies are serious pest on economically important vegetables in Asia. However, there are many taxonomic impediments when discriminating *Zeugodacus* species using their morphological taxonomic characters. The present study was thus aimed to differentiate three *Zeugodacus* (*Z. cucurbitae*, *Z. caudatus*, and *Z. tau*) species using their wing shape variation by applying the wing geometric morphometric (canonical variates) analysis as a novel approach.

Methods: Fruit flies were captured using Cue-lure fruit fly traps. Among collected fruit fly samples, five male adult specimens of each *Z. cucurbitae*, *Z. caudatus*, and *Z. tau* species were used to analyze the wing shape variation. The separated right wing of each male fruit fly specimen was mounted on a microscopic slide and photographs were taken. Fifteen anatomically corresponding (homologous) landmarks on each picture of a wing were generated using the software (tpsdig264). A set of coordinate data was generated for all the specimens selected using the MorphoJv1.02E software and wing shape variation data were analyzed using the canonical variate analysis by the software IBM SPSS, version 25.

Results: According to the Canonical Variate Analysis, the variation of wing venation among *Z. cucurbitae*, *Z. caudatus*, and *Z. tau* is significantly different. Individuals were clearly reported as three separate clusters by species in the CVA scatter plot of CV1 (eigen value = 3.50) against CV2 (eigen value = 1.20).

Conclusions: *Zeugodacus cucurbitae*, *Z. caudatus*, and *Z. tau* are independent of their wing venation patterns. The present study validates the applicability of wing shape variation to discriminate *Zeugodacus cucurbitae*, *Z. caudatus*, and *Z. tau* through landmark-based geometric morphometric analysis in taxonomic studies.

Keywords: Cryptic speciation, fruit fly, landmarks, wing venation, *Zeugodacus*

Host use and phylogeny of African *Dacus* (Diptera: Tephritidae) inferred from genome-scale data

Lore Esselens¹, Domingos Cugala², Maulid Mwatawala³,
Massimiliano Virgilio¹, Marc De Meyer¹

¹Royal Museum for Central Africa (RMCA), Tervuren, Belgium; ²Eduardo Mondlane University (EMU), Maputo, Mozambique; ³Sokoine University of Agriculture (SUA), Morogoro, Tanzania. E-mail: lore.esselens@africamuseum.be

Background: The genus *Dacus* Fabricius, 1805 (Diptera: Tephritidae) comprises more than 270 species, most of which occur in the Afrotropical region and include several economically important agricultural pests. The phylogenetic relationships within *Dacus* and the evolutionary history of host plant specialization remain incompletely resolved. Previous Sanger-based phylogenies suggested an association between host plant use and phylogenetic structure, but were constrained by limited marker resolution and taxon sampling. Here, we apply genome-scale data to reconstruct nuclear and mitochondrial phylogenies of African *Dacus* and to place these phylogenetic relationships in a broader evolutionary and temporal context.

Methods: We analysed 179 specimens representing 51 African *Dacus* species, a substantially expanded sampling compared to previous study (71 specimens from 32 species). Whole-genome sequencing was performed on both newly extracted material and long-term preserved DNA vouchers. Phylogenetic inference was based on orthologous groups identified using OMA standalone, with sequence reconstruction performed using the Read2Tree pipeline. Maximum-likelihood phylogenies were inferred from concatenated nuclear and mitochondrial nucleotide datasets using IQ-TREE2. Divergence times were estimated using a reduced set of conserved orthologous groups and fossil-calibrated molecular clock analyses implemented in MCMCtree.

Results: Genome-scale nuclear phylogenies recovered strongly supported clades corresponding to currently recognised *Dacus* species, providing robust validation of species boundaries and genus-level relationships. Major lineages showed consistent clustering by host plant family, with Apocynaceae-associated taxa forming a well-supported monophyletic group and Passifloraceae feeders nested within a broader paraphyletic group of Cucurbitaceae-associated species. Mitochondrial phylogenies were largely congruent with nuclear results, although with lower support and minor topological differences. The analyses revealed increased lineage structure within *Dacus humeralis*, with three distinct clusters instead of the two previously recognised. Fossil-calibrated divergence time estimates, incorporating representatives of major Dacini lineages, placed *Dacus* diversification within a broader temporal framework.

Conclusions: By combining expanded taxon sampling with genome-scale data, our study provides a robust and refined phylogenetic framework for African *Dacus*. The results support host-associated diversification, validate earlier phylogenetic hypotheses with substantially increased resolution, and reveal previously unrecognised lineage complexity within key taxa. These findings demonstrate the power of phylogenomics for resolving evolutionary relationships in species-rich insect groups and provide a foundation for future taxonomic, ecological, and pest-management studies.

Key words: true fruit flies, *Dacus*, phylogeny, orthologous genes

Session 3: Genetics and Biotechnology

**Progress toward developing ReMOT Control gene editing applications for the fruitflies
Ceratitis capitata and *Bactrocera dorsalis*.**

Anna Malacrida, Paolo Gabrieli, Alessandro Di Cosimo, Riccardo Piccinno, Giulia Fiorenza, Guliano Gasperi, Federico Forneris, Jason L. Rasgon

Department of Animal Biology and Biotechnology, University of Pavia, Pavia; Italy
Department of Entomology, The Pennsylvania State University, USA

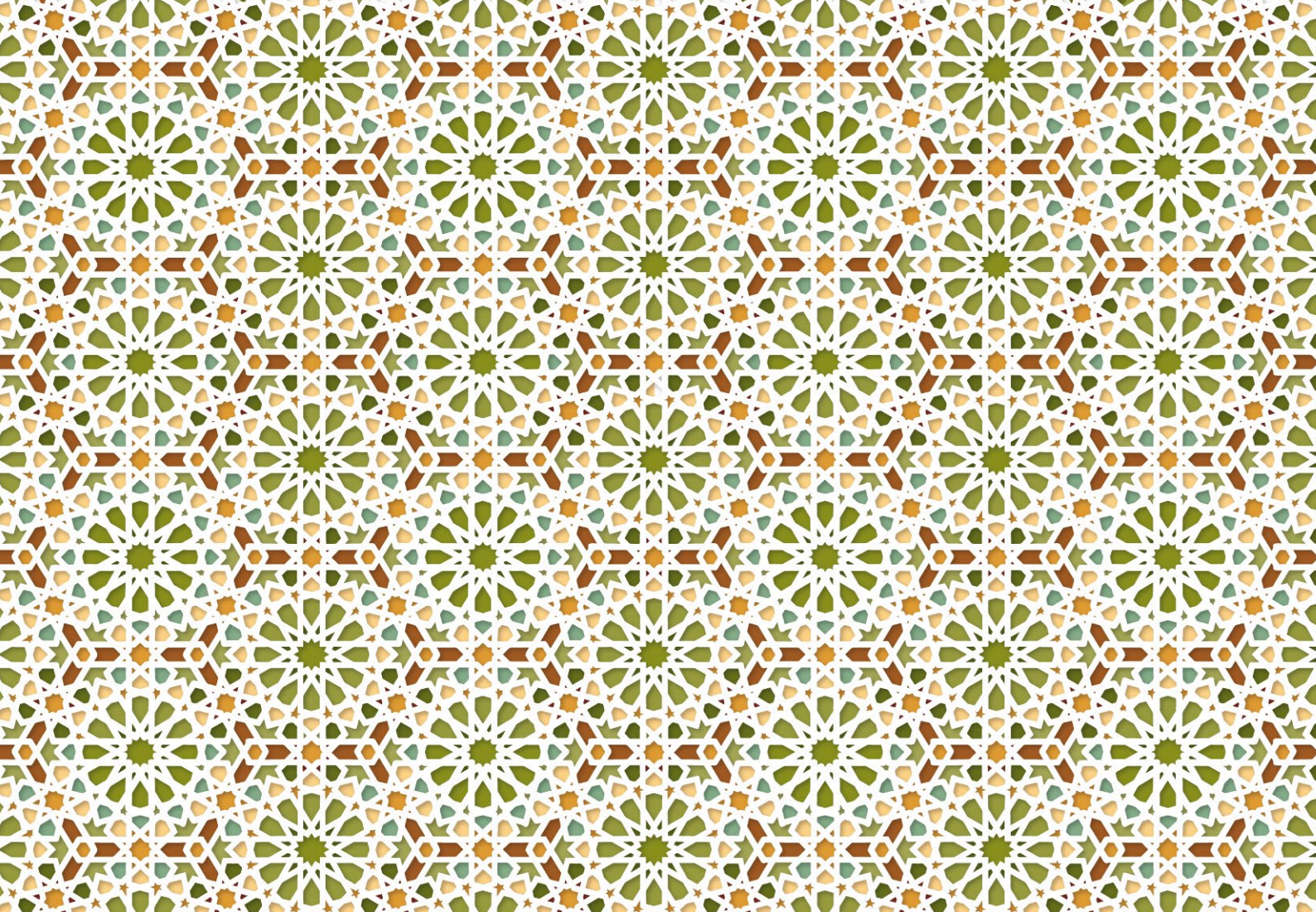
Background: Despite their importance, genetic manipulation of the fruitflies *Ceratitis capitata* and *Bactrocera dorsalis* are challenging due to technical and cost-related issues. Microinjection of gene-editing constructs into early stage embryos have previously been used for transgenesis, but this process is difficult and requires costly equipment. The discovery of Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein (CRISPR-Cas) technologies allowed for targeted mutagenesis and the creation of knockouts or knock-ins in many organisms. Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control) acts as an alternative to embryonic microinjections. ReMOT Control's main advantage is its ease of use coupled with the ability to hypothetically target any vitellogenic species. Indeed vitellogenins provide a natural ovarian delivery system to deliver Cas9 RNPs from the circulatory system to the developing oocytes of vitellogenic adult females, resulting in the transformation and modification of the germline of multiple species. Here, we assessed whether endogenous vitellogenins could be used to efficiently deliver molecular cargo into the ovary of *C. capitata* and *B. dorsalis*.

Methods: Three vitellogenin genes (*VG1*, *VG2*, and *VG3*) of *C. capitata* were identified, their tissue expression quantified, and their sequences cloned. The sequences were fused to EGFP, expressed, and their ovarian internalization assessed in adult vitellogenic females after injection into the hemolymph. We also tested their ability to enter the ovaries of the oriental fruitfly *Bactrocera dorsalis*.

Results: Two hours post-injection, recombinant CcVG2-EGFP was shown to be internalized into the ovaries of *C. capitata*, was observable in the yolk granules of mature ovaries at 24 hours post-injection, and was observable in oviposited eggs 48 hours post-injection. Similar patterns were observed in *B. dorsalis*.

Conclusions: These results provide proof-of-concept validation that CcVG2 is a promising candidate to target gene editing cargo to the developing germline of *C. capitata* and *B. dorsalis*. This opens opportunities for the development and optimization of a ReMOT Control gene editing approach in these species. The fact that CcVG2 is functional in *B. dorsalis* also has interesting implications for the evolution of ovarian receptors in fruitflies.

Key words: *Ceratitis capitata*, *Bactrocera dorsalis*, Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control), vitellogenins



POSTERS

Genetics and Biotechnology

OMICS-BASED EXPLORATION OF SYMBIONTS ASSOCIATED WITH *ANASTREPHA FRATERCULUS*

Claudia A. Conte¹, Romina M. Russo¹, Máximo Rivarola², Sergio González³, Fabián Milla¹, María C. Soria¹, María C. Giardini¹, Diego F. Segura^{1,4}, Albert Handler⁵, Kostas Bourtzis⁶, Joannis Ragoussis⁷, & Silvia B. Lanzavecchia¹

¹Instituto de Genética GV-IABIMO UEDD CONICET-INTA, Instituto Nacional de Tecnología Agropecuaria, Aristizabal and El Ñandú, 1686, Hurlingham, Buenos Aires, Argentina. ²Apolo Biotech-CONICET, Santa Fe, Argentina. ³Instituto de Agrobiotecnología y Biología Molecular (IABIMO) CONICET-INTA, Buenos Aires, Argentina. ⁴Instituto de Investigación en Ciencias Agrarias y Veterinarias, USAL, Argentina. ⁵U.S. Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, USA. ⁶Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna, Austria. ⁷McGill Centre, Montreal, Canada. E-mail: conte.claudia@inta.gob.ar

Background: *Anastrepha fraterculus* (Wiedemann 1830) is one of the most damaging pests of commercial fruit production in Argentina. Management of this quarantine pest relies mainly on chemical insecticides and trapping, although environmentally friendly strategies are increasingly adopted, including biological control with the parasitoid *Diachasmimorpha longicaudata* (Ashmead 1950) and the sterile insect technique (SIT). Climate change models predict a further expansion of its geographic range across the Americas, increasing its economic and phytosanitary relevance. Characterizing the microbial and viral communities of *A. fraterculus* may reveal targets for sustainable control strategies.

Methods: We performed an integrated metagenomic and metatranscriptomic analysis of *A. fraterculus* using Illumina and Oxford Nanopore sequencing. Metagenomic data were obtained from a single adult male and female, while metatranscriptomic data were generated from *D. longicaudata*-parasitized and non-parasitized larvae. Non-host reads were taxonomically classified using Kraken2 and subjected to comparative analyses.

Results: Metagenomic analysis revealed a diverse microbial community dominated by the endosymbiont *Wolbachia*, enabling recovery of its complete circularized genome (~1.46 Mb) and partial genomic sequences from other bacterial and fungal taxa. Viral sequences were detected exclusively in the female sample. Metatranscriptomic analyses showed differences in microbial and viral transcriptional activity between parasitized and non-parasitized larvae. *Wolbachia* displayed similar relative abundance under both larval conditions, whereas reads corresponding to a parasitoid-associated virus (DLEPV) were detected only in parasitized larvae. Other microbial taxa showed no significant differences between treatments.

Conclusions: This integrated approach identifies *Wolbachia* as a stable and dominant symbiont across larval and adult stages of *A. fraterculus* and reveals a specific association between parasitism and the presence of a parasitoid-associated virus at the larval stage. These findings provide a framework for future research aimed at strengthening sustainable fruit fly management strategies.

Key words: *Anastrepha fraterculus*, *Wolbachia*, *Diachasmimorpha longicaudata*, DLEPV

OBP genes expression in wild *Anastrepha ludens* females exposed to *Citrus aurantium* and *Mangifera indica* L. cv Ataulfo

Francisco García-Medina¹, Wendy Aragón-Gómez¹, Pablo Liedo² & Dina Orozco-Dávila^{1,2*}

¹Instituto de Biociencias, Universidad Autónoma de Chiapas, Blvd. Príncipe Akishino s/n, Tapachula 30798, Chiapas, México

²Departamento de Ecología de Artrópodos y Manejo de Plagas, El Colegio de la Frontera Sur (ECOSUR), Carretera Antiguo Aeropuerto Km 2.5, Tapachula 30700, Chiapas, México E-mail: dina.orozco@unach.mx

Background: In Mexico, *Anastrepha ludens* (Loew) is considered one of the most economically significant fruit fly pests due to the direct damage it causes to several fruit crops, as well as the phytosanitary restrictions associated with its presence. Losses in citrus and mango production can range from 10% to 25%. Highly valuable crops such as Ataulfo mango (*Mangifera indica*) are among the affected hosts. Odorant-binding proteins (OBPs) play a key role in the olfactory perception of insects and are essential in host location and selection. However, studies on *OBP* gene expression in *A. ludens* in response to different host fruits are still limited.

Methods: Expression levels of selected *OBP* genes were assessed using RT-qPCR, based on cDNA synthesized from total RNA samples. Wild, mated *A. ludens* females were exposed for six hours to either bitter orange or Ataulfo mango fruits. Gene expression was quantified using the comparative Ct method, with normalization against the *A. ludens* actin gene as an internal reference. Statistical analysis was performed using Student's t-test, with a significance level set at $p < 0.05$.

Results: Gene expression analysis revealed significant differential expression of all four *OBP* genes studied in flies exposed to bitter orange compared to those exposed to Ataulfo mango. This expression pattern was consistent with the infestation indices obtained previously, which were higher in bitter orange, with an index of 4.24. These findings suggest that the evaluated *OBP* genes are involved in host odor discrimination and may influence host selection behavior in *A. ludens*.

Conclusions: There appears to be a relationship between *OBP* gene expression and host preference in *A. ludens*. While increased expression of genes related to volatile compound detection may be associated with host preference potentially reflected in the number of larvae found in the field these findings should not be interpreted as definitive evidence. Larval presence and abundance may be influenced by multiple factors not evaluated in this study. Future research should include the use of specific volatile compounds and additional host fruit species to better understand host selection mechanisms.

Keywords: Tephritidae, *Anastrepha ludens*, host selection, *OBP* genes, gene expression.

Gut microbiota of *Drosophila suzukii* isolated from different hosts

Dina Orozco-Davila^{1,2}, Daniela Ruiz¹, Pablo Liedo² & Linnet Roque²

¹Instituto de Biociencias, Universidad Autónoma de Chiapas, Blvd. Príncipe Akishino s/n, Tapachula 30798, Chiapas, México

²Departamento Ecología de Artrópodos y Manejo de Plagas, El Colegio de la Frontera Sur (ECOSUR), Carretera Antigua Aeropuerto Km 2.5, Tapachula 30700, Chiapas, México. E-mail: dina.orozco@unach.mx

Background. The gut microbiota of polyphagous insects can vary depending on the host plant, directly influencing various aspects of their biology, such as nutrition, development, and environmental adaptation. *Drosophila suzukii* is an invasive pest of major economic importance due to its ability to infest fruits with thin skins, including strawberry, blackberry, grape, blueberry, and raspberry. In Mexico, this species has been reported in nine federal states; however, there are still regions free of this pest that possess suitable climatic conditions for its establishment and proliferation. Given that *D. suzukii* attacks a wide variety of fruit crops and that its feeding and reproductive habits are closely linked to the environment, studying the relationship between the host plant and gut microbiota becomes relevant. Understanding these interactions may contribute to the development of more effective management strategies.

Methods: Young adults of *Drosophila suzukii*, both wild and laboratory-reared, were used in a starved state. The guts of these individuals were dissected, from the foregut to the hindgut, for DNA extraction. The V3–V4 region of the 16S ribosomal RNA gene was amplified and sequenced using the Illumina NovaSeq platform to compare the gut bacterial community of flies fed a laboratory diet with those fed blackberry and raspberry fruits.

Results. A total of eight phyla, 50 classes, 144 orders, 243 families, and 436 bacterial genera were identified. The phylum *Proteobacteria* was dominant across all treatments, and the *Acetobacteraceae* family, belonging to the group of acetic acid bacteria, showed the highest relative abundance. The genus *Gluconacetobacter* predominated in wild flies and was also detected in one laboratory replicate (L3). Unidentified bacteria from the family *Comamonadaceae* were found in flies from blackberry, while *Acetobacter* was more abundant in laboratory flies. The genus *Ralstonia* was detected exclusively in this treatment. Alpha diversity was higher in laboratory flies, which showed greater bacterial richness compared to wild flies. In general, the gut bacterial communities displayed dominance of few taxa across all treatments, with relatively higher evenness in laboratory flies.

Conclusions. The gut bacterial communities of *Drosophila suzukii* is dominated by acetic acid bacteria regardless of the origin of the flies. However, differences in alpha diversity among treatments indicate that diet influences the organization of these communities, suggesting a plastic microbiota capable of adapting to different dietary contexts.

Keywords: Spotted wing, 16S rRNA, bacterial community, symbiosis, Illumina, host

Integrating DNA barcoding and ortholog-based genomics to improve diagnostics for *Dacus frontalis*

Fanny Kratz, Lore Esselens, Abir Hafsi, Pia Addison, Domingos Cugala, Maulid Mwatawala, Ghania Barech, Mourad Khaldi, Jochem Bonte, Nathalie Smits, Hélène Delatte, Marc De Meyer, Wannes Dermauw, Massimiliano Virgilio

Royal Museum for Central Africa (RMCA), Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo), Leuvensesteenweg ,13, 3080, Tervuren, Belgium.
fanny.kratz@africamuseum.be

Background: *Dacus frontalis* (*Df*) is an economically significant fruit fly species widely distributed across Africa and already intercepted in the European Union (EU), making accurate diagnostics essential for trade and phytosanitary control. Coordinated sampling with African partner institutions has revealed unusually high mitochondrial divergence of the cytochrome c oxidase subunit I (COI) gene - about 4% - between North African and sub-Saharan populations. This level of intraspecific variation complicates species identification and highlights the need for a more geographically representative molecular reference framework, as well as tools capable of resolving population structure to support origin tracing in case of new incursions.

Methods: To improve diagnostic reliability, we are expanding continental COI barcode coverage by generating new Sanger sequences and extracting mitochondrial barcodes from short-read genomic datasets. In parallel, we are establishing an ortholog-based genomic workflow using Orthologous Matrix (OMA) standalone to identify orthologous genes and Read2Tree to reconstruct phylogenomic datasets. Although still in development, this genomic framework is designed to reveal population structure at a broader genomic scale and will support future origin-tracing analyses should additional interceptions occur.

Results: Current COI data confirm the presence of two well-differentiated mitochondrial lineages within *Df*, corresponding broadly to northern and sub-Saharan regions. Expansion of the barcode dataset is improving geographic representation and refining our understanding of this divergence. The ortholog-based component is being assembled and will be used to explore genome-wide differentiation and lineage structure once additional data are available.

Conclusions: Building an integrated molecular framework combining expanded COI coverage with an emerging ortholog-based genomic approach will strengthen diagnostic capacity for *Df*. By enabling more robust identification and providing a foundation for future origin tracing, this work supports improved surveillance and phytosanitary preparedness - both within Africa and in regions where the species poses an incursion risk, including the EU.

Key words: Phytosanitary control, identification, origin tracing, population structure, *Dacus frontalis*

VIRAL DIVERSITY IN *ANASTREPHA FRATERCULUS*

Jéssica Fernandes Padre¹, Sabrina Ferreira de Santana¹, Lucas Yago Melo Ferreira¹,
Jonatha dos Santos Silva¹, Ramon Costa Dominato¹, Eric Roberto Guimarães Rocha
Aguar¹, Reinaldo A. de Brito², **Janisete Gomes Silva¹**

1 – Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rodovia Jorge Amado, Km 16. CEP: 45662-900 Ilhéus, BA, Brazil; 2 – Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos, SP, Brazil. E-mail: jgs10@uol.com.br

Background: The *Anastrepha fraterculus* species complex is among Brazil's most economically important fruit flies. The identification of the virus diversity (virome) allows to track possible emergent viruses and to understand host-pathogen interactions. In this study we aimed to characterize the virome associated with the nominal species *A. fraterculus*.

Methods: 39 publicly available transcriptome libraries of *Anastrepha fraterculus* were retrieved from the NCBI Sequence Read Archive and analyzed using a metatranscriptomic workflow. Raw sequencing data were subjected to quality control procedures to remove low-quality reads and sequencing artifacts. High-quality reads were subsequently mapped against the host reference genome using Bowtie2 to reduce host background and enrich for non-host sequences. The remaining reads were *de novo* assembled using a combined strategy based on SPAdes and Trinity to maximize viral contig recovery. Putative viral sequences were characterized through sequence similarity searches against the NCBI nucleotide (NT) and non-redundant protein (NR) databases. Evolutionary relationships were inferred using maximum-likelihood phylogenetic reconstructions implemented in IQ-TREE based on manually curated MAFFT alignments.

Results: Virome analysis revealed a diverse viral community comprising positive-sense single-stranded RNA viruses (ssRNA+, n = 11), negative-sense single-stranded RNA viruses (ssRNA–, n = 3), and double-stranded RNA viruses (dsRNA, n = 1). Among the identified viruses, four corresponded to previously described taxa, including two negev-like viruses (99% identity), a crupavirus (97%), and a Nora virus (99%). Additionally, five viral sequences showed high similarity to viruses recently detected by our group in *A. obliqua* but not yet formally published, including noda-like viruses (93% and 99% identity), an iflavirus (98%), and a sigmavirus (99%), indicating they share a core virome. Notably, the sigmavirus was experimentally validated using specific primers and detected in both male and female individuals collected from cajá and umbu, supporting its natural association with this host.

Conclusions: Overall, these findings reveal an extensive and largely uncharacterized viral diversity associated with this species, suggesting that its virome may play an important role in host biology, potentially influencing fitness, physiology, and ecological interactions. The identification of these viruses also provides a foundation for future studies exploring their potential use in biological control strategies.

Key words: Virome, *Anastrepha*, metatranscriptome, South American fruit fly

UNPRECEDENTED VIRAL DIVERSITY IN *ANASTREPHA OBLIQUA* UNVEILED BY RNA-SEQ

Jéssica Fernandes Padre ¹, Sabrina Ferreira de Santana ¹, Lucas Yago Melo Ferreira ¹,
Jonatha dos Santos Silva¹, Ramon Costa Dominato¹, Eric Roberto Guimarães Rocha
Aguiar ¹, Reinaldo A. de Brito ², **Janisete Gomes Silva¹**

1 – Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rodovia Jorge Amado, Km 16. CEP: 45662-900 Ilhéus, BA, Brazil; 2 – Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos, SP, Brazil. E-mail: jgs10@uol.com.br

Background: *Anastrepha obliqua* is widely distributed in the Neotropical region and is one of the fruit fly species of the most economic importance in Brazil. Tephritids can be infected by viruses whether they show symptoms or not. Viromics, employing the strategy of transcriptome sequencing, have proved useful to identify the diversity of viruses present in these organisms and the information recovered is valuable not only to track possible emergent viruses that could threat food security, but also to understand host-pathogen interactions and the impact of the pathogen in natural populations of fruit flies. Yet, knowledge on viromes associated to tephritid fruit flies is still scarce and no viruses have been reported for species in the genus *Anastrepha*. The objective of this study was to characterize the virome associated with *A. obliqua*.

Methods: We analyzed 23 transcriptome libraries of *A. obliqua* available at the NCBI SRA database. Raw reads were quality-trimmed and assessed prior to the removal of host-derived sequences based on the reference genome. The remaining reads were *de novo* assembled, and viral contigs were identified through similarity searches against reference Nucleotide and Protein databases, followed by confirmation and taxonomic assignment using updated BLAST analyses. Selected viral sequences were further validated by RT-PCR using virus-specific primers.

Results: Using this strategy it was possible to identify 13 previously undescribed viral genomes associated with *A. obliqua*, encompassing all major viral genome classes, including +ssRNA (n = 5), –ssRNA (n = 3), dsRNA (n = 1), and RT viruses (n = 1). Most genomes were complete or near-complete and exhibited high divergence at the protein level, with RdRp sequence identities ranging from approximately 29% to 85%. Selected viruses were experimentally validated by RT-PCR, confirming the presence of viral RNA in independent samples belonging to Nodaviridae, Phasmaviridae, Phenuiviridae, and Rhabdoviridae.

Conclusions: These findings reveal a highly diverse viroma associated with this insect pest and highlight public RNA-seq datasets as a valuable resource for viral discovery, with potential implications for host biology. Further studies are necessary to understand the real viral diversity within *Anastrepha*, which viruses are exclusive of each species, possible emergence as threats of plant species, and their impact on fruit flies.

Key words: Virome, *Anastrepha*, metatranscriptome, West Indian fruit fly

***Lactobacillus kunkeei*: A bacterium with potential benefits for *Anastrepha obliqua* (Diptera: Tephritidae)**

Linnet Roque-Romero^{1*}, Karina Guillén-Navarro¹, Eugenia Zarza^{1,2}, Pablo Montoya³, Pablo Liedo¹

¹El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km 2.5, Tapachula, Chiapas, 30700 México

²Investigadora por México, Consejo Nacional de Humanidades Ciencias y Tecnologías Insurgentes Sur 1582, 03940 Ciudad de México, México

³Instituto de Biotecnología, Universidad Autónoma de Chiapas, Boulevard Akichino S/N, 30798, Tapachula, Chiapas, México

E-mail: linnet.roque@estudianteposgrado.ecosur.mx

Background: Supplying mass-reared *Anastrepha obliqua* with gut bacteria may reduce the gap in sexual competitiveness between wild and laboratory flies. Because native gut bacteria are mainly enterobacteria with potential human pathogenicity, we evaluated the effects of the safe bacterium *Lactobacillus kunkeei* on *A. obliqua* biological traits.

Methods: We performed tests of sexual competitiveness and survival under starvation with flies fed with sugar diet, standard diet (3:1 sugar: protein), sugar + *L. kunkeei*, and standard diet + *L. kunkeei*.

Results: Consumption of *L. kunkeei* improved the sexual competitiveness of *A. obliqua* males fed with sugar. We did not find statistical differences between the treatments of standard diet, standard diet + *L. kunkeei*, and sugar diet + *L. kunkeei* in the sexual competitiveness tests, while males fed only with sugar were the least favored.

Females and males of *A. obliqua* fed with the sugar diets showed increased survival compared to flies fed with standard diet. The addition of *L. kunkeei* resulted in an increased life expectancy of females and males compared to the treatments without the bacterium.

Conclusions: Our results suggest that *L. kunkeei* may be a bacterium with potential benefits for *A. obliqua*.

Key words: West Indian fruit fly, Probiotic, Sterile Insect Technique, Mass rearing, Sexual competitiveness, Survival

Urban-scale connectivity and subtle temporal genetic changes in *Bactrocera zonata* populations from Tel Aviv

Sam Vanbergen^{1,2}, Lore Esselens¹, Pablo Deschepper^{1,3}, Michael Ben-Yosef⁴, Marc De Meyer¹, Massimiliano Virgilio¹

1: Invertebrate Section, Royal Museum for Central Africa (RMCA), Leuvensesteenweg 13, 3080 Tervuren, Belgium, E-mail: massimiliano.virgilio@africamuseum.be

2: Biology Department, Ecology, Evolution and Conservation Biology, Biology Department, KU Leuven, 3000 Leuven, Belgium, E-mail: sam.vanbergen@kuleuven.be

3: Biodiversity & Ecosystems Data & Information Centre (BEDIC), Royal Belgian Institute of Natural Sciences (RBINS), Vautierstraat 29, 1000 Brussels, Belgium, E-mail: pablo.deschepper@naturalsciences.be

4: Agricultural Research Organization (ARO), Gilat Center, M.P. Negev 8531100, Israel, E-mail: michaelb@volcani.agri.gov.il-

Background: *Bactrocera zonata* is an invasive fruit fly of quarantine concern in the Middle East, yet its population connectivity in urban environments remains poorly understood. Within the REACT project, we characterized the local genetic structure and temporal dynamics of *B. zonata* populations in Tel Aviv to assess dispersal patterns and evidence for secondary introductions.

Methods: Adult males were sampled using methyl-eugenol traps at nine urban and peri-urban sites across Tel Aviv in 2023 and 2024, with historical samples from 2020 included for comparison. Genome-wide SNP data were generated using whole-genome resequencing, and mitochondrial haplotypes were reconstructed. Population structure was investigated using PCA, admixture analyses, DAPC, pairwise F_{ST} , and isolation-by-distance tests, and local patterns were interpreted in a regional genomic context.

Results: Overall, spatial genetic differentiation within Tel Aviv was negligible. PCA, admixture, and mitochondrial analyses revealed extensive overlap among sites and years, and pairwise F_{ST} values were uniformly low, indicating a largely panmictic urban population. However, DAPC and diversity metrics revealed subtle temporal signals, including shifts in cluster composition and a significant reduction in observed heterozygosity between 2023 and 2024, suggesting ongoing demographic or genetic changes.

Conclusions: Comparisons with regional populations allowed us to exclude recent secondary introductions from several well-characterized regional populations, including India, Iran, Oman, Pakistan, and the Mascarenes. Nevertheless, limited genomic data from neighboring countries prevent a full assessment of regional connectivity.

These results highlight the high dispersal capacity of *B. zonata* in urban landscapes and emphasize the need for broader regional surveillance to support effective management strategies.

Key words: *Bactrocera zonata*, peach fruit fly, population genetics, dispersal

High connectivity and genetic stability of *Bactrocera dorsalis* in La Réunion revealed by genome-wide analyses

Sam Vanbergen^{1,2}, Lore Esselens¹, Pablo Deschepper^{1,3}, Marc De Meyer¹, Massimiliano Virgilio¹, Hélène Delatte⁴

1: Invertebrate Section, Royal Museum for Central Africa (RMCA), Leuvensesteenweg 13, 3080 Tervuren, Belgium, E-mail: massimiliano.virgilio@africamuseum.be

2: Biology Department, Ecology, Evolution and Conservation Biology, Biology Department, KU Leuven, 3000 Leuven, Belgium, E-mail: sam.vanbergen@kuleuven.be

3: Biodiversity & Ecosystems Data & Information Centre (BEDIC), Royal Belgian Institute of Natural Sciences (RBINS), Vautierstraat 29, 1000 Brussels, Belgium, E-mail: pablo.deschepper@naturalsciences.be

4: CIRAD-UMR PVBMT, 3P, 7 chemin de l'IRAT, 97410 Saint Pierre, La Réunion, E-mail: helene.delatte@cirad.fr

Background: The oriental fruit fly, *Bactrocera dorsalis*, is a highly invasive agricultural pest whose establishment on islands poses major challenges for containment and long-term management. Within the EU-funded REACT project, we investigated the local population genetic structure and short-term temporal dynamics of *B. dorsalis* in La Réunion to assess connectivity and potential secondary introductions.

Methods: Adult males were sampled using methyl-eugenol traps across ten semi-urban sites spanning wide altitudinal gradients. Sampling was conducted in two periods (2023 and 2024). Genome-wide SNP data were generated through short-read whole-genome sequencing, complemented by mitochondrial haplotype reconstruction. Population structure was assessed using PCA, admixture analyses, DAPC, pairwise *F*_{ST}, and isolation-by-distance tests, and results were interpreted within a regional genomic framework.

Results: All analyses consistently revealed a lack of spatial or temporal genetic structure across the island. Pairwise *F*_{ST} values were extremely low, mitochondrial haplotypes showed no spatial clustering, and no isolation by distance or altitude was detected. Genetic diversity metrics, including nucleotide diversity, heterozygosity, and inbreeding coefficients, were stable across sites and years. Comparisons with regional datasets, including La Réunion individuals from 2019, confirmed that La Réunion populations belong to the Mascarene genomic cluster and provided no evidence for recent secondary introductions from Africa or Asia.

Conclusions: From a management perspective, the lack of detectable population structure indicates high connectivity across La Réunion, highlighting the importance of considering island-scale processes in surveillance and management planning.

Key words: *Bactrocera dorsalis*, oriental fruit fly, population genetics, dispersal

***Bactrocera oleae* gut microbiome as a resource to improve insect mass rearing**

Aya Elsayed¹, Silvia Romagnolo¹, Kostas Bourtzis², Alberto Alma¹, Elena Gonella¹

¹ Department of Agricultural, Forest and Food Sciences, University of Torino, Italy

² Insect Pest Control Subprogramme, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, P.O. Box 100, 1400 Vienna, Austria

E-mail: aya.elsayed@unito.it

Background: Insect diversity and evolutionary success are significantly influenced by their interactions with beneficial microorganisms. The gut microbiome is essential for nutrition, digestion, immunity, and overall health in insects. The olive fruit fly, *Bactrocera oleae* (Rossi), a major pest in olive cultivation, has a gut microbiome dominated by the symbiont '*Candidatus* *Erwinia dacicola*'. Laboratory rearing conditions lead to symbiont loss, reducing insect fitness critical for sterile insect technique (SIT) programs.

Methods: We conducted transcriptomic sequencing of the gut microbiome in a field-collected population and its lab-reared F1 generation to identify differentially expressed genes (DEGs) in response to the shift from natural to artificial diet; additionally, taxonomy assignment was conducted to determine microbial composition of the insect guts, and relevant bacteria were isolated in pure cultures. Since a slight reduction of *E. dacicola* was recorded in the lab-reared generation, the long-term symbiont loss was simulated through antimicrobial treatment, and the performance of treated flies was compared to that of control flies. Moreover, qPCR was employed to compare the abundance of active *E. dacicola* with and without anti-symbiont treatment.

Results: Our results confirmed that *E. dacicola* is a core member of the olive fruit fly gut microbiome, as its reduction resulted in DEGs linked to nutrient metabolism, stress response, and immune function. Antimicrobial treatment significantly reduced the abundance of *E. dacicola* and the fly performance. Moreover, the *E. dacicola* decrease in the gut microbial composition of the F1 generation corresponded to a replacement by other taxa, suggesting a compensatory role. A total of 103 common metabolic pathways were identified between the main symbiont and the bacterial isolates. *Stenotrophomonas* isolates exhibited augmented coenzyme A production pathways, whereas *Enterobacter* isolates had superior carbohydrate processing capabilities. To test this hypothesis, we assessed the impact of redelivering isolated gut bacteria, which were abundant in the F1 microbiome, on long-term lab-reared *B. oleae*, by incorporating these microbes into the fly diet. Several microbial isolates were shown to increase the fly performance.

Conclusions: These findings underscore the dynamic nature of the olive fruit fly gut microbiome and the critical role of *E. dacicola* in maintaining its balance. Additionally, functionally equivalent culturable bacteria may serve as substitutes for the uncultivable obligate symbiont *Ca. E. dacicola* to improve insect performance.

Key words: *Bactrocera oleae*, Gut microbiome, Insect's fitness, *E. dacicola*

Insights on the structure, organization and regulation of Tephritid sex chromosomes

Dimitris Rallis¹

¹ University of Thessaly

Background: Sex chromosomes and the mechanisms underlying their evolution across lineages represent a long-standing and intriguing field of study. Driven by sexual reproduction and the need for sex determination, sex chromosome systems have evolved independently in multiple organisms, yet often display convergent characteristics. In lineages where heteromorphic XY sex chromosomes exist, the Y chromosome is typically highly repetitive, degenerated, and gene poor, while the X-chromosome usually evolves dosage compensation mechanisms to balance the hemizygous X state in XY males. Despite these shared characteristics, the gene repertoire and expression dynamics of sex chromosomes might vary among different lineages. In *Drosophila melanogaster*, the model species of Diptera, its typical morphology of heteromorphic XY chromosomes is mirrored in many Tephritidae species, like *Ceratitis capitata*. However, the sex chromosome-linked genes vary between these two lineages, with *D. melanogaster* X-linked genes appearing autosomal in *C. capitata* and vice versa. This divergence underscores the dynamic evolution of sex chromosomes and questions the suitability of *D. melanogaster* as a model for sex chromosome biology.

Methods: To elucidate this, we investigate the structure, organization and regulatory mechanisms of Tephritid sex chromosomes. We employ sexed single embryo RNA-seq to detect *C. capitata* early embryonic expression patterns, where we also observe signals of active dosage compensation on X-linked genes. We further extend the analysis to adult stages where we perform RNAi-mediated characterization of known dosage compensation mediators to assess their chromosome-wide regulatory impact. In parallel, we analyze the structure and organization of the poorly studied Tephritid Y chromosomes, to elucidate its origin, evolutionary relation and divergence across Tephritid species.

Development of a temperature-sensitive lethal strain and Y chromosome targeting in the oriental fruit fly *Bactrocera dorsalis*

Giovanni Petrucci^{1,2}, Marc F. Schetelig^{2,3}, Kostas Bourtzis¹

¹Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna, Austria. ²Department of Insect Biotechnology in Plant Protection, Justus-Liebig-University Gießen, Institute for Insect Biotechnology, Gießen, Germany. ³Liebig Centre for Agroecology and Climate Impact Research, International Atomic Energy Agency Collaborating Centre, Justus Liebig University Gießen, Gießen, Germany

E-mail: G.Petrucci@iaea.org

Background: The oriental fruit fly, *Bactrocera dorsalis*, is a well-known generalist agricultural pest that is now widely distributed across the world. As such, is a primary target of Sterile Insect Technique (SIT) programmes. Genetic Sexing Strains (GSS) play a crucial role in improving both the efficiency and costs of SIT. In these strains, males and females carry differently expressed phenotypic markers, such as different puparium colour, to allow easy separation. While GSSs have traditionally been developed via chemical- or irradiation-induced chromosomal translocations, CRISPR-based genome editing can now be used to accelerate development. As a phenotypically visible marker, white pupae (wp) mutants have been generated in several tephritid species, including *B. dorsalis*. Moreover, the previously unknown genetic background of the temperature-sensitive lethal (*tsl*) marker in *Ceratitis capitata* has been identified, where a single non-synonymous SNP in the *lysine--tRNA ligase* (*Lysyl-tRNA synthetase*, *LysRS*) gene caused the heat-dependent phenotype. However, this marker has not yet been developed in any GSS strain of *B. dorsalis*, which rely exclusively on pupal colour for sex separation. For GSS development, CRISPR-based knock-in could be used to target Y-chromosome-specific regions, allowing the introduction of the rescue allele of the desired marker without the need for chromosomal translocation.

Methods: We targeted the *LysRS* gene in *B. dorsalis* to induce a single point mutation via CRISPR/Cas9, which would produce the same aminoacidic substitution that was proven to be the causal mutation for the *tsl* phenotype in *C. capitata*. Moreover, we identified via bioinformatic analysis several putative Y specific loci as possible targets for CRISPR-mediated knock-in, which we then proceeded to validate via PCR and sequencing.

Results: After injection and non-lethal genotyping, we identified two families carrying the desired SNP in the *LysRS* gene in *B. dorsalis*, as well as six families with a knockout mutation. We validated and sequenced three loci along with their flanking regions on the Y chromosome, confirming their male-specificity.

Conclusions: The induced mutation in the candidate gene is a promising advance in the development of new *tsl* based GSS in *B. dorsalis*. The characterization of multiple Y-specific regions provides a first step toward the development of CRISPR-based GSS in this species.

Key words: Genetic sexing strain, *LysRS*, white pupae, CRISPR/Cas9, genome editing

COMPARATIVE PERFORMANCE OF ZINGERONE AND RASPBERRY KETONE IN THE MONITORING OF AFRICAN *DACUS*

Happy Leonard¹, Laila Timana², Inocêncio Lopes², Laura Canhanga^{2,3}, Domingos Cugala^{2,3}, De Meyer, Marc⁴, Ramadhani Majubwa¹, Abdul Kudra¹, Alcardo.Alex¹, Aruna Manrakhan⁵ & Virgilio, Massimiliano⁴

¹Department of Crop Science and Horticulture, Sokoine University of Agriculture (SUA), Morogoro, Tanzania; ²Faculty of Agronomy and Forest Engineering, Eduardo Mondlane University, Maputo, Mozambique; ³Centre of Excellence in Agri-Food Systems and Nutrition, Eduardo Mondlane University, Maputo, Mozambique; ⁴Biology Department, Royal Museum for Central Africa, Tervuren, Belgium; ⁵Citrus Research International, Nelspruit, South Africa. E-mail: happyleonard97@gmail.com

Background: This study builds on previous work evaluating trapping systems for monitoring Afrotropical fruit flies, particularly the comparative assessment of lure types and trap designs conducted by Manrakhan *et al.* (2017). While that study provided a baseline for the efficacy of available trapping systems, important knowledge gaps remain regarding the optimisation of male lures for *Dacus* species, especially in terms of lure formulation, dose, and release characteristics under field conditions. The present study represents a follow-up effort, focusing specifically on the performance of alternative formulations of zingerone and raspberry ketone, tested through harmonised field trials in Mozambique and Tanzania.

Methods: Field trials are being conducted using 32 traps per country, deployed weekly across three different farms, and comprising six lure treatments and two shared controls represented by traps baited with torula yeast. Three zingerone-based treatments compared a melted formulation without carrier and carrier-based formulations using liquid paraffin or propylene glycol as release retarders, each delivering 2.28 g of zingerone per trap. Three raspberry ketone treatments compared two ethanol-based doses (1.88 g and 2.50 g per trap) and a formulation combining ethanol and propylene glycol as a release retarder (1.88 g per trap). Each treatment included four traps, with one dental roll and vapona per trap. The minimum distance between traps is 50 m, and catches are collected weekly and used to quantify species composition and the relative attraction of males between different formulations.

Results: Field sampling is currently ongoing in both Mozambique and Tanzania. To date, the coordinated experimental design has generated comparable datasets across multiple farms and sampling periods, enabling preliminary assessment of trap performance and species composition. Ongoing collections are providing data on male attraction to different lure formulations, including comparisons among lure concentrations and carrier-mediated release strategies. Final analyses will quantify differences in capture rates and species-specific responses once all sampling rounds are completed.

Conclusions: This ongoing study provides a harmonised framework to evaluate lure formulation, dose, and release characteristics for *Dacus* monitoring under African field conditions, strengthening regional fruit fly surveillance programmes.

Keywords: chemical attractants, tephritidae, monitoring, male lures.

CYTOGENETIC CHARACTERIZATION OF LABORATORY POPULATIONS OF THE *ANASTREPHA FRATERCULUS* CRYPTIC SPECIES COMPLEX

M. Cecilia Giardini¹, Mariela Nieves², Elena Cancio-Martinez³, Fabián H. Milla¹, M. Florencia Fourastié⁴, Graciela González⁴, M. Teresa Vera³, Kostas Bourtzis³ & Silvia B. Lanzavecchia¹

¹Laboratorio de Genética y Genómica de Insectos, Instituto de Genética, Instituto Nacional de Tecnología Agropecuaria, Argentina. E-mail: giardini.maria@inta.gob.ar;

²Laboratorio de Arquitectura Genómica de Mamíferos-CEMIC Saavedra, Unidad Ejecutora CONICET, Argentina; ³Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna, Austria; ⁴Laboratorio de Citogenética y Evolución, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina, Instituto de Ecología, Genética y Evolución de Buenos Aires, CONICET–UBA, Argentina.

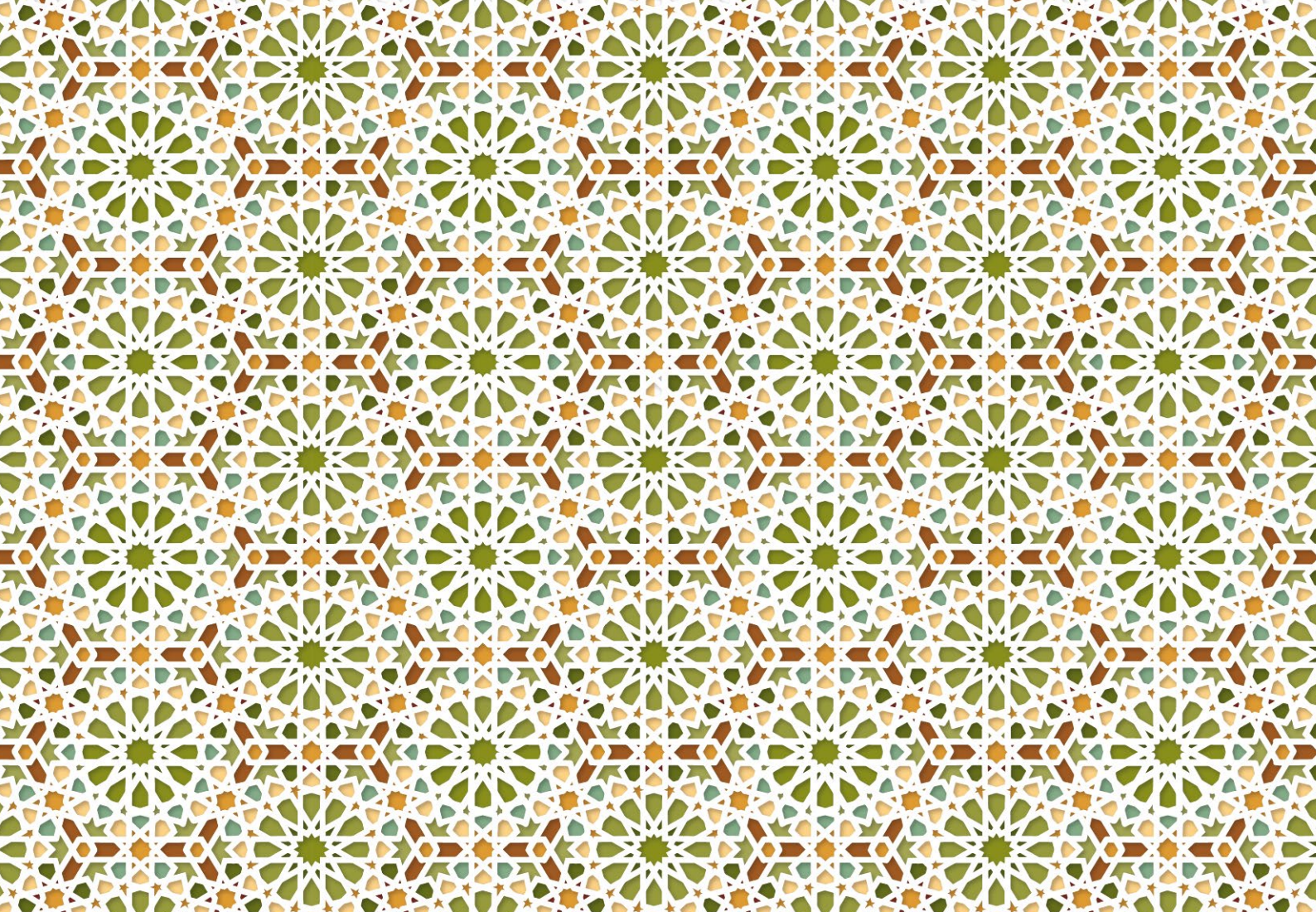
Background: The South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae), is a major agricultural pest and represents a cryptic species complex. Eight morphotypes, initially identified based on morphological traits and latter supported by genetic and behavioural data, are distributed from Mexico throughout South America to northern and central Argentina. Cytogenetic analyses have proven useful for detecting chromosomal polymorphisms in both laboratory strains and wild populations. This information is particularly relevant because laboratory colonies often constitute the foundational material for developing genetic sexing strains (GSS), and chromosomal variation may affect the performance of novel strains used in sterile insect technique (SIT) applications. Six of the eight *A. fraterculus* morphotypes have been cytogenetically characterized (Andean, Peruvian, Ecuadorian, and Brazilian-1, -2, and -3). The aim of this study was to describe the karyotype of *A. fraterculus* laboratory populations maintained at the Insect Pest Control Laboratory (IPCL) and compare them with previously reported cytogenetically defined morphotypes.

Methods: We analyzed IPCL laboratory populations from Vacaria (Brazil), Piracicaba (Brazil), Piura (Peru), Cusco (Peru), Ibagué (Colombia), and Guayaquil (Ecuador). Mitotic chromosome preparations were obtained from cerebral ganglia of third-instar larvae. At least 10 individuals per population were studied. Slides were prepared and sequentially stained with DAPI/CMA and examined under a light microscope. An average of 15 metaphases per slide were scored.

Results: Cytogenetic analysis identified two novel karyotypes in the Piracicaba (Brazil) and Cusco (Peru) strains of *A. fraterculus*. Populations from Guayaquil (Ecuador) and Piura (Peru) shared a karyotype matching the Ecuadorian, Peruvian, or sp.4.

Conclusions: These novel karyotypes reveal the hidden diversity within the *A. fraterculus* cryptic species complex and underscores the need for routine karyotypic characterization of colonies used in SIT-related research. The shared karyotype among geographically distinct populations may reflect either a wider distribution of this morphotype or convergence under laboratory conditions. These findings emphasize the importance of integrating cytogenetic analysis with genetic, behavioural, and field-based studies to accurately delimit morphotypes and to ensure the development of stable and effective GSS for SIT applications.

Key words: South American fruit fly, karyotype, cytology, morphotypes



POSTERS

Chemical Ecology and Attractants

Optimization of Mass Trapping of *Drosophila suzukii* on Blueberry in Northwestern Morocco: Comparative Evaluation of Fermentative Baits and Renewal Intervals

Background: The expansion of blueberry production (*Vaccinium* spp.) in Morocco is increasingly threatened by the spotted-wing drosophila, *Drosophila suzukii* (Matsumura, 1931), an invasive pest able to infest healthy ripening fruits, resulting in significant economic losses and export quality constraints. Within Integrated Pest Management (IPM), mass trapping is a key approach for monitoring and suppressing adult populations, particularly ovipositing females. However, trap efficiency depends strongly on the attractiveness of fermentative baits and the frequency of lure renewal, especially under warm agroclimatic conditions.

Methods: A comparative field study was conducted from 30 April to 2 June 2025 in two greenhouse blueberry farms located in northwestern Morocco: Moulay Bousselham (Ventura cultivar) and Sidi Yahya El Gharb (Sekoya Pop cultivar). Using DROSOSAN commercial traps, two homemade fermentative attractants were tested at equal volume (360 mL): DL (yeast + sugar + water) and DV (yeast + wheat flour + vinegar + apple cider vinegar). Each attractant was evaluated under three renewal intervals (every 3, 7, and 15 days) in a randomized complete block design with four replicates per treatment. Captured insects were collected, preserved, and identified to assess total catches, sex ratio, and ecological selectivity (non-target captures). Data were analyzed using two-way ANOVA followed by SNK post-hoc tests ($p < 0.05$).

Results: Renewal frequency was the most critical factor affecting trap performance in both sites ($p < 0.010$ at Moulay Bousselham; $p < 0.001$ at Sidi Yahya El Gharb). The 3-day renewal interval consistently resulted in significantly higher captures compared to 7- and 15-day intervals. At Sidi Yahya El Gharb, DV renewed every 3 days achieved the highest performance (353.7 adults/trap), whereas at Moulay Bousselham, DL renewed every 3 days was superior (174.95 adults/trap), highlighting an inter-site divergence at optimal renewal. Trapping was female-biased in all treatments; notably, DL showed a significantly higher proportion of females at Sidi Yahya El Gharb (95.54%; $p = 0.033$). Regarding selectivity, DV attracted significantly more non-target arthropods (Coleoptera and Hymenoptera) than DL in both locations ($p < 0.021$), and this effect increased with longer renewal intervals.

Conclusions: This study confirms that frequent renewal of fermentative baits is essential to maximize *D. suzukii* mass trapping effectiveness under Moroccan greenhouse conditions. Although DV may provide high short-term attractiveness under stable climates, DL renewed every 3 days offers the best overall compromise by ensuring high capture efficiency, strong female selectivity, and reduced impact on non-target beneficial arthropods. These findings support the standardization of DL with a 3-day renewal interval as an optimized protocol for sustainable IPM strategies against *D. suzukii* in Moroccan blueberry production systems.

Key words: *Drosophila suzukii*, blueberry, mass trapping, fermentative attractants, renewal interval, selectivity, sex ratio, IPM, Morocco

Methyl eugenol response by *Bactrocera dorsalis* males is suppressed by low semiochemical doses in the adult diet

G. I. González-López¹, Kevin Malod¹, Tania Pogue^{1,2}, **Christopher W. Weldon**^{1,2}

¹Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

²Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Hatfield 0028, Pretoria, South Africa

E-mail: cwweldon@zoology.up.ac.za

Background: The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a major pest of fruit production worldwide. Control strategies include the male annihilation technique (MAT), which uses the strong male attractant methyl eugenol (ME), and the sterile insect technique (SIT). However, simultaneous application of MAT and SIT is hindered by sterile males being attracted to and killed by ME-baited devices. A potential solution is to reduce *B. dorsalis* male response to ME through dietary supplementation prior to release. In this study, we evaluated the effects of four semiochemicals (ME, eugenol, trans-cinnamic acid, and zingerone) incorporated into the adult diet at different concentrations on male response to ME baited traps under semi-field conditions.

Methods: Each semiochemical was incorporated at concentrations of 5, 2.5, 1.25, or 0% (control) into a 3:1 sugar-hydrolysed yeast mixture. Virgin males were fed these diets for 48 h at different ages (2, 8, 18 days after adult emergence). After supplementation, groups of 25 males per treatment were released into field cages with citrus trees and two bucket traps, one baited with ME and one unbaited, both containing dichlorvos insecticide blocks. Releases were carried out at four times of day, and each was observed for 90 minutes.

Results: Dietary supplementation with 1.25% ME or eugenol suppressed male captures in ME-baited traps across all age groups. Suppression was strongest in 4-day-old males and remained consistent at 10 and 20 days of age. The response to the traps peaked between 10:00 and 15:00, with reduced captures in the early morning and late afternoon, indicating that age and time of day modulate responsiveness even under effective suppression.

Conclusion: Males fed low concentrations (1.25%) of ME or eugenol prior to released showed suppressed responses to ME-baited traps. This enables the simultaneous application of MAT and SIT, with eugenol emerging as a viable alternative to ME.

Key words: Semiochemical, methyl eugenol, pre-release diet, male annihilation technique (MAT), sterile insect technique (SIT)

ATTRACTION OF *Anastrepha ludens* TO VOLATILE COMPOUNDS EMITTED BY FIVE MANGO CULTIVARS

Judith Velazquez^{1,2}, Dina Orozco^{1,2}, Julio Rojas², Leopoldo Cruz-López², **Pablo Liedo**²

¹*Instituto de Biociencias, Universidad Autónoma de Chiapas. Boulevard Príncipe Akishino s/n, Col. Solidaridad 2000, Tapachula 30798, Chiapas, México.*

²*Departamento de Ecología de Artrópodos y Manejo de Plagas. El Colegio de la Frontera Sur (ECOSUR). Carretera Antigua Aeropuerto, Tapachula 30700, Chiapas, México. E-mail: pliedo@ecosur.mx*

Background: Mango (*Mangifera indica* L.) is one of the most widely consumed fruits worldwide due to its nutrient and characteristic flavor and aroma, which are primarily determined by its volatile compounds. The Mexican fruit fly, *Anastrepha ludens*, is considered a species of high economic importance and is classified as a polyphagous pest, as it infests a wide variety of host plants. To develop more effective trapping systems for monitoring and managing fruit flies, it is essential to understand their resource-searching behavior and identify the chemical and visual cues that influence it. The objective of this research was to determine the attraction of *A. ludens* to mixtures of selected volatile compounds emitted by five mango cultivars.

Methods: Field cage tests and electroantennography (EAG) assays were conducted using mass-reared, non-irradiated flies aged 11–14 days. The field cage assays were performed in successive phases. In the first phase, mixtures derived from five cultivars were compared. The mixture of the cultivar that elicited the highest attraction was then selected, and blends containing different numbers of compounds (nine, six, four, three, and binary mixtures) were formulated and evaluated in subsequent phases.

Results: The mixture derived from the Kent cultivar captured significantly more flies than the other treatments. Two mixtures formulated from compounds present in the Kent blend were found to be as attractive as the complete mixture. The mixture of the three major compounds (myrcene, 3-carene, and β -caryophyllene) offers a promising alternative for developing a more effective attractant for *Anastrepha ludens*. Electroantennography (EAG) assays confirmed that both females and males exhibited the highest antennal responses to the same treatments: the Kent mixture, the blend of the three major compounds, the mixture lacking 3-carene, and the blend of the four minor compounds.

Conclusion: Our results indicate that although neither the Kent volatiles blend nor the mixture of the three major compounds can match Ceratrap in attraction efficacy, these mixtures show promise for the development of a semiochemical-based attractant for *A. ludens*.

Key words: Tephritids, Mexican fruit fly, synthetic mixtures, kairomones.

α -Copaene lures: More potent, longer lasting & lower cost than Trimedlure plugs for monitoring *Ceratitis capitata*.

Saida Kharrat¹, Todd Shelly², Cam Oehlschlager³, Rick Kurashima², Carolina Calvo³, Carlos Rodriguez³, Erica Gonzalez-puschendorf³, & Francisco Gonzalez-Fuentes³,

¹Department of Life Sciences, University of Carthage, Bizerte, 7021, Tunisia

²USDA-APHIS, Kapolei, HI, USA

³Chemtica Internacional, Calle El Beneficio, Sto Domingo, Heredia, Costa Rica

Background: In 2020 a process was developed that converted a plentiful commercially available natural oil to ~90% pure α -copaene. Initial trials in Costa Rica and Tunisia revealed that Dome traps baited with sachets loaded with 4.2 g of this α -copaene that were not replaced during a year-long trial, outperformed traps baited with 2-g trimedlure (TML) plugs replaced at 6-week intervals. A 20-week trial using Jackson traps baited with the same sachet of α -copaene outperformed Jackson traps baited with 2-g of TML on a dental wick renewed weekly or 4-g TML plugs. Neither the α -copaene lures nor the 4-g TML plugs were replaced during the trial.

Methods: A new dispenser, a plastic bulb designed to fit in the lure-holding basket of a Jackson trap, was developed and tested in an 18-week trial in Tunisia, a 20-week trial in Costa Rica and a 24-week trial in Hawaii. Traps baited with the basket-fitting α -copaene lures (3 or 4 g a.i.) were not replaced during the trial.

Results: During only 3 of the 62 weeks of these trials did TML baited traps capture numerically more *Ceratitis capitata* than those baited with α -copaene. In the Tunisian and Costa Rican trials control traps were baited with 2-g TML on a dental wick renewed weekly. In the Hawaiian trial fresh and field aged 4-g TML plugs were used.

Conclusions: α -Copaene basket fitting lures (3 and 4-g a.i.) are less expensive than 4-g TML plugs and give satisfactory performance vs 2-g TML on a dental wick renewed weekly for 12 weeks.

Key words: *Ceratitis capitata*, trimedlure, α -copaene, monitoring, area-wide

Vial-lure as attractant for *Anastrepha* surveillance on mango orchards in Chiapas, México

Salvador Flores¹, Sergio Campos¹, Pablo Montoya^{2,3}, Jorge Toledo³, Pablo Liedo³, Antonio Villaseñor⁴, Walther Enkerlin⁵

¹Programa Operativo de Moscas, SADER-SENASICA, Metapa de Domínguez, Chiapas, Mexico; ²Instituto de Biociencias, Universidad Autónoma de Chiapas, S/N, Tapachula, Chiapas, México; ³El Colegio de la Frontera Sur, Tapachula, Chiapas, México; ⁴Asesor independiente, Nayarit, México; ⁵Insect Pest Control Subprogramme, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Austria. E-mail: salvador.flores.i@senasica.gob.mx

Background: *Anastrepha ludens* is a quarantine pest of mango, limiting the marketing and export of the fruit to pest-free areas. In the Soconusco region of Chiapas, Mexico, the sterile insect technique (SIT) is used as a tool within a systems approach that has facilitated fruit exports. Efficient integrated pest management requires early detection of the pest, making it necessary to evaluate new products that can reduce pest management costs. A new synthetic food attractant, Vial lure (Beter world®), formulated as cylindrical vial lures (12 mm diameter; 30 mm height), consisting of ammonium acetate and putrescine has been developed to be used in surveillance tephritid programs. It new attractant was compared against torula yeast borax (TYB) as standard bait.

Methods: A mango cv. Ataulfo orchard (14° 53'01"N, 92°13'20"W) in Tapachula, Chiapas was selected to evaluate three attractants: 1) TYB in Multilure trap was prepared by suspending 3 TYB pellet (5 g each; Scentry Biologicals Inc., Billings, MT, USA) per 300 mL water per trap. 2) Vial lure in Multilure were affixed to the inner top surface of the trap; insects were retained in a 20% propylene glycol solution. 3) Vial lure in the Jackson trap were suspended from the metal hanger positioned along the apex of the trap; a sticky insert retained the insects entering. According to IAEA standardized trapping protocol, a randomized block design was used, with five plots each containing one trap per treatment. TYB was replaced after trap service, and Vial lure was not replaced during the two-months study. Wild and sterile specimens of *Anastrepha ludens*, as well as wild *A. serpentina*, were quantified.

Results: The vial attractant captured 304 wild adults, 57 sterile adults of *A. ludens*, and 80 adults of *A. serpentina*. For wild *A. ludens*, the average capture rate was significantly lower in the JT/Vial 2C treatment, but the proportion of females did not vary significantly among the treatments. The capture of sterile adults of *A. ludens* did not vary significantly among the three treatments. For *A. serpentina*, no differences were between the Vialure treatment in the Multilure trap and the TYB treatment, while in the Jackson trap, the Vial attractant did not attract adults of this species.

Conclusions: Vial baits represent an alternative to TYB for monitoring *Anastrepha ludens* wild populations in mango orchards, lengthening the bait replacement interval and reducing supply and labor costs. In addition, vial lure can be used for SIT proposes, in monitoring the dispersal of released sterile males.

Key words: *Anastrepha ludens*, *A. serpentina*, mangoes, Multilure trap, torula yeast

Session 4. Chemical Ecology and Attractants

LOWER THE TRAP HEIGHT, HIGHER THE CATCHES: A CASE OF SYMPATRIC FRUIT FLIES ATTRACTED TO BLENDS OF POPULAR PARA-PHEROMONES

Kashinath Chiluwal¹*, Sunil Aryal², Salina Magar³, Ajaya S.R. Bajracharya¹

¹ National Entomology Research Centre, Khumal Complex, Lalitpur, Nepal

² Nepal Agricultural Research Council, Singhadurbar Plaza, Kathmandu, Nepal

³ Himalayan College of Agricultural Science and Technology, Kathmandu, Nepal

E-mail: Knchilu@gmail.com, chilukn@gmail.com

Background: Nepal is facing a growing challenge of tephritid fruit flies in cucurbit cropping systems. Availability of several tephritid fruit fly species sensitive both towards popularly used para-pheromones; cue lure (CL) and methyl eugenol (ME), and the cue-loaded trap installation heights are not the issues perceived by the cucurbit growers resulting into the repeated seasonal losses of cucurbit fruits to the flies. So, the tephritid-complex is a must-address issue of Nepalese cucurbit farming communities.

Methods: Firstly, the bottle traps fitted inside with the paper releasers ($\phi = 2$ cm) impregnated with 250 μ L total volume of cues (ME, CL or ME+CL) were installed at three height strata (40, 80 and 120 cm) above ground surface. Secondly, the six different blending ratios of ME: CL (100: 00, 75:25, 50:50, 25:75, 0: 100 and 00:00) were evaluated. All the experiments were evaluated inside the research station, at out-reach research (OR) sites and at the farmer's fields. The trapped fruit flies were identified using pictorial-keys, the mean proportionate trap-catches both for the trapping height and for blending ratios were separated using one-way analysis of variance followed by a range test, the Tukey's HSD (Honestly Significant Different) test at $P = 0.05$.

Results: Four CL-sensitive [(*Zeugodacus tau* Walker, *Zeugodacus cucurbitae* (Coquillett), *Zeugodacus scutellaris* (Bezzi) and *Dacus longicornis* Wiedemann)] and three ME-sensitive [(*Zeugodacus diversus* (Coquillett), *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders))] fruit fly species were trapped in the traps; highly significant proportion (67.42%) being attracted to the traps fitted above 40 cm above ground surface followed by those fitted at 80 cm (20.95%) and 120 cm (11.63%) irrespective of the species and the attractants. Similar pattern of attraction in OR site (61.93, 57.58 and 10.49% respectively at traps fitted at 40, 80 and 120 cm above ground) and farmer's field (64.23, 24.57 and 11.20%, respectively) were recorded. Out of the total collected 36,332 individuals, the dominant species was *Z. tau* with Berger-Parker dominance index of 0.55 followed by *B. dorsalis* (0.17) and *Z. cucurbitae* (0.11). The flies were highly attractive to their respective sensitive cues. However, the availability of many sympatric species in cucurbit cropping system demands for the blend of ME: CL. The 25ME: 75CL was found effective in attractive higher number of species and individuals, exhibiting a future prospect of developing an effective composite lure.

Conclusions: The fruit fly management practices in Nepal should target *Z. tau*, as it is found to dominate the sympatric tephritid flies in cucurbit cropping system. The releasers baited with 250 μ L of 25: 75 blends of ME and CL could be deployed in the traps fitted near the ground surface as it is proven through this study that "Lower the trap height, higher the catches".

Key words: Cucurbits, Cue lure, Fruit flies, Methyl eugenol, Trap height

EVALUATION OF THE POTENTIAL OF CITRUS ESSENTIAL OILS FOR TRAP ATTRACTIVENESS AGAINST *DROSOPHILA SUZUKII* UNDER GREENHOUSE AND FIELD CONDITIONS

Hasnae Ennouinou^{1,2,3}, Said Zantar⁴, Yousra Zantar^{4,5}, Aouatif Benali⁶, Fouad Mokrini⁶, Lhoussaine El Rhaffari⁷, Abdelmalek Joutei Boutaleb², Rachid Lahlali^{2,x},
Khalid Khfif^{1,*,x}

¹Laboratory of Entomology, Research Unit on Nuclear Techniques, Environment, and Quality, National Institute for Agricultural Research (INRA), 78 Bd. Sidi Med. Ben Abdellah, 90010 Tangier, Morocco

²Department of Plant Protection, National School of Agriculture of Meknes, Km10, Rte Haj Kaddour, BP S/40, Meknes, 50001, Morocco

³Plant Protection Unit, Hassan II Agronomic and Veterinary Institute, Rabat, Morocco

⁴Research Unit on Nuclear Techniques, Environment and Quality, National Institute of Agronomic Research (INRA), Tangier, Morocco

⁵Faculty of Science and Technology, Abdelmalk Saidi University, Tangier, Morocco

⁶Biotechnology Unit, National Institute for Agricultural Research, Rabat, Morocco

⁷Laboratory of Bio-Actives Health and Environment, Faculty of Sciences, Moulay Ismail University of Meknes, Meknes, Morocco

*Corresponding author: khalid.khfif@inra.ma

^xAuthors contributed equally to this work.

Background: Globally, *Drosophila suzukii* is recognized a major invasive pest that causes serious economic losses to soft-skinned fruit production. In Morocco, it damages strawberries, blackberries, raspberries, and blueberries. Females lay eggs in ripening fruits, which limits the effectiveness of control strategies. Pest control relies mainly on insecticides, which can lead to resistance, environmental pollution, and non-target effects on beneficials.

Methods: This work evaluated citrus essential oils as alternatives to synthetic products for the management of *D. suzukii*. Nine formulations (F1–F9) prepared from essential oils of dried bitter orange (*Citrus aurantium*) flowers, fresh orange (*Citrus sinensis*) zest, and fresh lemon (*Citrus limon*) zest were tested at three doses under greenhouse and field conditions.

Results: In the greenhouse, the formulation F1, containing the lowest dose of fresh orange zest essential oil, captured twice as many females (5 ± 0.71) as the control (2.5 ± 0.65). Field trials conducted in a raspberry plantation in Larache, Morocco, confirmed this trend, with F1 showing the highest female captures (20.33 ± 3.38), significantly higher than the positive control (9.33 ± 4.81).

Conclusions: These results indicate that citrus essential oils, particularly low-dose of fresh orange zest essential oil, can be developed as attractant tools to support trapping programs for *D. suzukii*.

Key words: *Drosophila suzukii*, Essential oil, Formulation, Biocontrol, Attractiveness.

Cue Lure-Mediated Male Annihilation in Fruit Flies: Insights into Attractant Efficacy across Tropical and Temperate Regions

Anjana G. S., Sajad Hussain Mir

Division of Entomology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Wadura, Sopore -193201, Jammu & Kashmir, India.

E-mail: drsajadento@skuastkashmir.ac.in

Background: Fruit flies (Diptera: Tephritidae) are serious pests of cucurbitaceous crops, causing substantial yield losses under diverse agro-climatic conditions. The Male Annihilation Technique (MAT) using cue lure is an eco-friendly management strategy; however, its field efficacy depends on lure dosage, trap servicing frequency and visual attributes of traps. The present study aimed to optimize MAT parameters under temperate and tropical conditions of India.

Methods: Field experiments were conducted during Kharif 2024 at Wadura, Kashmir (SMW 28-37) and Rabi 2024-25 at Palakkad, Kerala (SMW 49-6). The effects of cue lure quantity (0.1-1.0 ml), frequency of lure recharging (weekly to four-weekly) and trap colour were evaluated using transparent bottle traps and modified Steiner traps under Randomized Block Design. Fruit fly catches were recorded weekly, pooled species-wise and analysed using one-way ANOVA at $p \leq 0.05$.

Results: Significant differences ($p \leq 0.05$) were observed among cue lure quantities. In Kashmir, traps charged with 1.0 ml cue lure recorded the highest mean catch of *Zeugodacus cucurbitae* (8.05 ± 0.20 flies/trap/week, SEM), significantly superior to lower doses ($CD_{0.05} = 0.589$). In Kerala, the same treatment resulted in maximum captures of *Z. cucurbitae* (11.32 ± 0.31 flies/trap/week) and *Z. tau* (7.35 ± 0.43 flies/trap/week) ($CD_{0.05} = 0.915$ and 1.28 , respectively). Weekly recharged traps were significantly more effective, recording 4.90 ± 0.56 flies/trap/week in Kashmir and 30.90 ± 0.85 (*Z. cucurbitae*) and 11.50 ± 0.98 (*Z. tau*) flies/trap/week in Kerala. Trap colour significantly influenced attraction, with yellow traps recording the highest catches in Kashmir (10.43 ± 0.65 flies/trap/week) and Kerala (19.20 ± 1.10 and 5.00 ± 0.80 flies/trap/week for *Z. cucurbitae* and *Z. tau*, respectively).

Conclusions: The study confirms that MAT efficacy against cucurbit fruit flies is significantly enhanced by optimizing lure dosage, servicing frequency and trap colour. A 1.0 ml cue lure dose, weekly recharging and yellow-coloured traps are recommended across agro-climatic regions. These findings provide scientifically validated guidelines for strengthening sustainable fruit fly management programmes.

Key words: Male annihilation technique, Cucurbitaceous crops, Cue lure, Trap colour, *Zeugodacus cucurbitae*, *Zeugodacus tau*

New Mass Trapping lure for the control of *Drosophila suzukii* (Diptera, Drosophilidae)

Shakir Al-Zaidi, Rihem Moujahed, Zinette Moussa & Harry Edwards

Russell IPM LTD, 45 First Avenue, Flintshire, Industrial Park, CH5 2NU, United Kingdom. E-mail: Shakir@russellipm.com

Background: The spotted wing drosophila (SWD), *Drosophila suzukii* (Diptera, Drosophilidae), is a major pest of soft fruits worldwide, causing serious losses to fruit production. In response to tighter restrictions on chemical pesticides, farmers are shifting towards integrated pest management (IPM), that includes physical and semiochemical approaches, such as traps and lures, to protect crops and reduce environmental impact. The effectiveness of liquid lures for mass-trapping SWD varies widely. Some commercial baits based on fermentation products may offer reasonable attractiveness to flies but with low selectivity, making SWD monitoring and identification time-consuming and difficult.

Methods: Two trials were conducted during summer 2025 in Kent, south-east England, to evaluate the new liquid lure formulation and new trap design (Maxlure) from Russell IPM LTD for mass trapping of SWD. The first trial was carried out on a raspberry farm and aimed to compare the efficiency and selectivity of Maxlure liquid with the top 4 leading lures on the UK market. The second trial was carried out on a strawberry farm and aimed to compare the efficiency of Maxlure Light Trap with the standard trap generally used by farmers in the region, as well as to assess the impact of light on SWD catch. In both trials, liquids were collected on a weekly basis. Trap contents were classified as male and female SWD, and other *Drosophila* species. In addition, female SWD were divided into mated females, mated females holding eggs and virgin females.

Results: Compared with the average catch of the 4 leading lures on the market, Maxlure caught 2.5 times more SWD. In Maxlure traps, less than 2.5% of the catch was from other *Drosophila* species, demonstrating that Maxlure is more attractive and more selective for SWD. Also, Maxlure attracted 4 times more females holding eggs ready to be laid in fresh fruit than the average of the 4 lures, thereby reducing the risk of females laying eggs in fruits and decreasing potential fruit damage. The light in the Maxlure trap increased capture of SWD by 160%, with a female-to-male ratio of 1.3: 1 and a 3-fold increase in the number of female SWD holding eggs compared with the standard traps.

Conclusion: This study demonstrated that the new Maxlure is more effective at controlling SWD and reducing fruit damage. Light in the Maxlure trap increased the capture rate of egg-bearing female SWD.

Key words: *Drosophila suzukii*, SWD, mass trapping, integrated pest management, soft fruits, Maxlure,

Evaluation of traps, attractants and insecticides against the apple fruit fly in tejocote orchards in central Mexico

Víctor M. Pinto, Raúl Nieto, Francisca Hernández & Samantha Robledo

Universidad Autónoma Chapingo, Chapingo, Texcoco, Estado de México 56230.

Email:vpinto@chapingo.mx

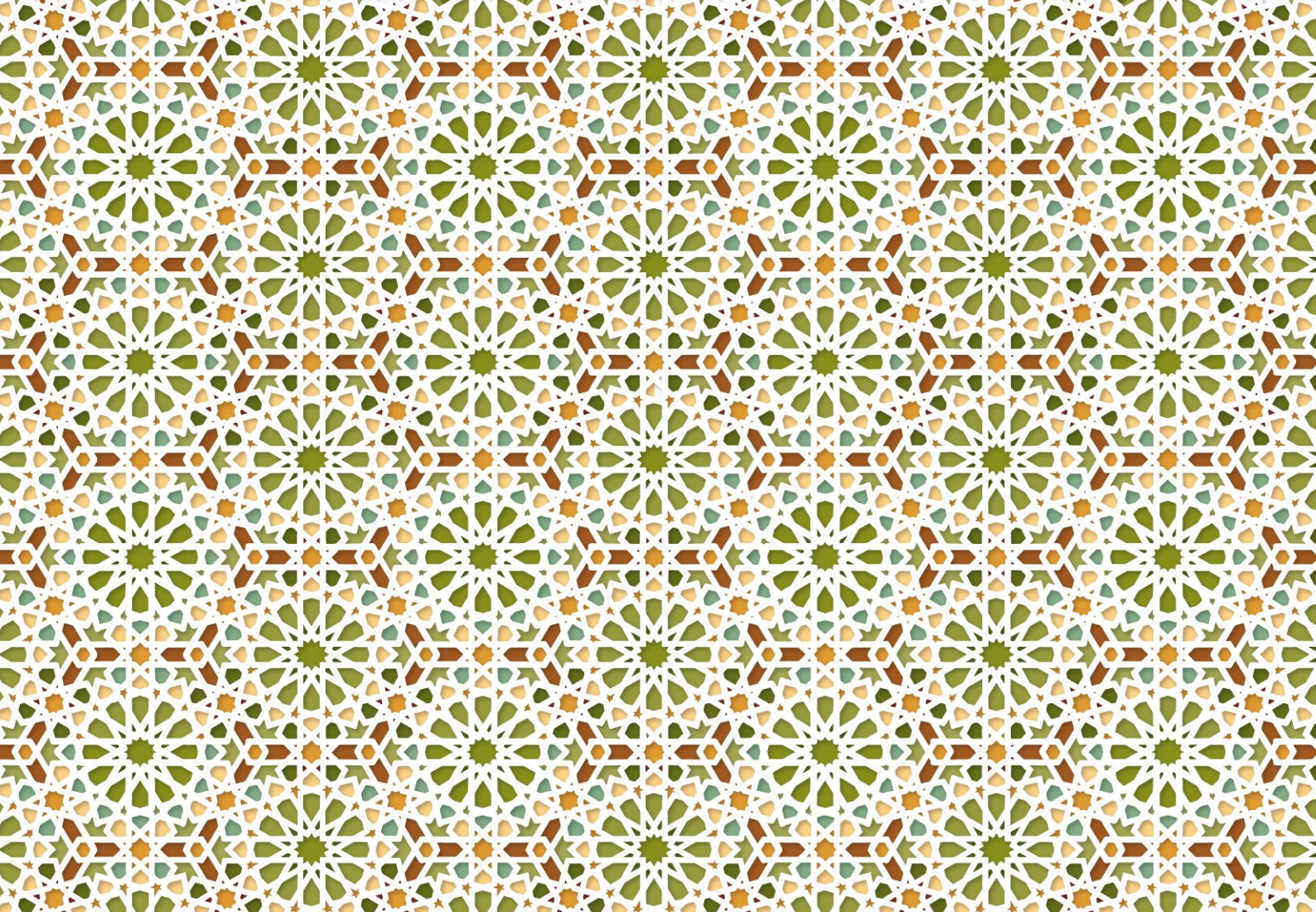
Background. Tejocote (*Crataegus mexicana* Moc y Sessé), a kind of hawthorn tree, is native to Mexico. Its fruit, with high pectin content, are commonly consumed in Mexico and USA during cultural and religious celebrations of winter season. In Mexico, the apple fruit fly, *Rhagoletis pomonella* (Walsh), has been a serious limiting factor for hawthorn production for many years. It diminishes fruit yield and quality, leading in some cases to the abandonment of plantations or cutting down of trees and their replacement with other fruit or ornamental species. The implementation of trapping as a chemical control strategy for this pest is one of the main control methods for minimizing damage by the pest.

Methods. The aim of this study was to evaluate the effectiveness of two shapes (spherical and flat panel) of sticky traps; four colors - yellow, orange, white, and red - of the traps and two food attractants (hydrolyzed proteins and Spinosad) for monitoring the flies. The experiment was conducted in orchards located at three locations - El Verde, Chiautzingo, Puebla and Texcoco, Estado de Mexico) in central Mexico. At Texcoco orchards the effectiveness of two insecticides - malathion and Spinosad - were evaluated on adult populations of *R. pomonella* through 11-weekly samplings: two before insecticide application (used as control) and nine thereafter.

Results. The results of multifactorial analysis showed that the yellow flat trap proved to be the best for trapping *R. pomonella* flies, with no significant differences in regard to attractant or location. Assessment of insecticides showed that both were effective against the pest, with no significant differences between them and populations declined to zero immediately after application.

Conclusions. The use of yellow flat traps is recommended as a reliable tool for monitoring and potentially reducing populations. Both malathion and spinosad can be used as viable chemical control options, depending on the grower's preferences (e.g., organic vs. conventional management). The combination of efficient monitoring and timely chemical control should reduce losses, improve fruit quality, and prevent orchard abandonment.

Key words: *Crataegus*, hawthorn, malathion, *Rhagoletis pomonella*, Spinosad



POSTERS

Risk Assessment, Quarantine and Post-harvest

Sperm in traps: assessment of female mating status to increase monitoring efficiency

Diana Pérez-Staples¹, Gisela Castillo², Solana Abraham², Betzabé Verónica-Murrieta¹, Abraham Moreno-Mejía¹, Aldebron Charlotte³, Kamala Jayanthi⁴, Jaipal S. Choudhary⁵, Nicholas Manoukis^{3,6}, Laura Moquet⁷, Corinna Bazelete⁸

¹INBIOTECA, Universidad Veracruzana, Xalapa, Veracruz, Mexico. Email: diperez@uv.mx ²Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales (LIEMEN), PROIMI-Biotecnología, CONICET, Tucumán, Argentina. ³USDA, Daniel K. Inouye US Pacific Basin Agricultural Research Center, Hilo, Hawaii. ⁴Indian Institute of Horticulture Research, Bangalore, Karnataka, India. ⁵ICAR RCER, Farming System Research Centre for Hill and Plateau Region, Plandu Ranchi, Jharkhand-834010, India. ⁶USDA Agricultural Research Service, European Biological Control Laboratory (EBCL), 810 avenue du Campus Agropolis 34980 Montpellier-sur-Lez, France. ⁷UMR PVBMT, CIRAD, Saint-Pierre, La Réunion, France. ⁸USDA APHIS PPQ Science and Technology Venture II, 920 Main Campus Drive, Cube 5A-15 Raleigh, NC 27606.

Background: The success of monitoring pest species is key to influencing mitigation decisions and management actions. Accurate determination of mating status has economic implications because enacting quarantines is expensive, as incorrect assessment can lead to costly and ineffective quarantine actions. Here, we determined the likelihood of correctly determining mating status in pestiferous tephritid fruit flies from traps, and how different baits and dyes could impact the correct assessment.

Methods: Mated and unmated *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha fraterculus*, *Ceratitis capitata*, *Bactrocera dorsalis*, *Bactrocera latifrons*, *Bactrocera zonata*, *Bactrocera tau*, and *Zeugodacus cucurbitae* were left in liquid baited traps for 7 days in outdoor conditions. We then dissected females and blindly determined their mating status. We identified which sperm storage organ (spermathecae or ventral receptacle (VR)) yielded the most accurate results, and for *B. dorsalis*, *B. latifrons*, *B. zonata*, and *B. tau* we evaluated the proportion of sperm stored in the VR.

Results: The type of bait used affected the correct assessment of mating status in *A. ludens* and *A. obliqua*, with traps baited with propylene glycol yielding the lowest correct results. Accuracy of mating status assessment was highest in traps baited with Ceratrap and Spinosad. Overall, it was easier to accurately detect an unmated than a mated female. For all species, accuracy was above 50% except for wild-mated *B. dorsalis* and *Z. cucurbitae*. We found the proportion of sperm stored in the VR varied from 10 to 80% depending on species.

Conclusions: Revision of the ventral receptacle is important. The use of aceto-orcein as dye did not improve the correct assessment of mating, while bait type only affected a few species. Alternative methodologies such as molecular techniques or machine learning could be implemented for more accurate determination of mating status, particularly for *B. dorsalis* and *Z. cucurbitae*.

Key words: Mating, *Bactrocera*, *Anastrepha*, *Ceratitis*, baits, sperm

Machine Learning-Based Ensemble Species Distribution Modelling of *Ceratitis cosyra* (Diptera: Tephritidae) in Africa: A climate-based baseline for risk mapping

Joseph Ruboha¹, Jenipher Tairo¹, Happy Leonard^{1,3}, Luis Bota⁴, Abdul Kudra¹, Ramadhani Majubwa¹, Alcadro Barakabitze⁴, Maulid Mwatawala¹, Marc De Meyer², and Massimiliano Virgilio²

1. Department of Crop Science and Horticulture, Sokoine University of Agriculture, Tanzania

2. Biology Department, Royal Museum for Central Africa, Tervuren, Belgium

3. Crop Protection Section, Tanzania Agricultural Research Institute (TARI), Morogoro, Tanzania

4. National Fruit Fly Laboratory, Chimoio, Mozambique

5. Department of Informatics and Information Technology, Sokoine University of Agriculture, Tanzania

*Corresponding email for the first author: joseph.ruboha@sua.ac.tz

Background

The mango fruit fly *Ceratitis cosyra* (Walker) is a major pest of mango, marula and other tropical fruits in sub-Saharan Africa, causing substantial yield losses and contributing to quarantine restrictions along regional and export value chains. Anticipating current and future risk requires spatially explicit tools that relate species occurrence to environmental conditions at continental scale.

Methods

As part of an ongoing PhD project, we developed a cross-validated machine learning-based ensemble species distribution model (SDM) for *C. cosyra* across Africa using high-resolution climate and elevation data. Georeferenced presence records were compiled from the RMCA database and DISPEST-supported surveys. Nineteen WorldClim v2.1 bioclimatic variables and elevation (2.5-arc-minute resolution) were screened for multicollinearity using variance inflation factors, resulting in a parsimonious set of five predictors (BIO4, BIO6, BIO13, BIO14, BIO18, BIO19, and Elevation). A presence-background framework was adopted, with spatial thinning applied to reduce clustering, yielding 157 presence and 6,336 background records for modelling.

Results

Six algorithms were fitted to the thinned dataset: MaxEnt (L1-regularised logistic regression), Random Forest (RF), Extreme Gradient Boosting (XGBoost), Adaptive Boosting (AdaBoost), Light Gradient Boosting Machine (LightGBM), and Artificial Neural Networks (ANN). Under spatial cross-validation, LightGBM and AdaBoost exhibited the highest discrimination (mean AUC = 0.720 and 0.719, respectively), followed by XGBoost (0.706) and RF (0.682), while MaxEnt (0.587) and ANN (0.594) performed less well. A cross-validation-weighted ensemble was constructed, assigning proportionally greater influence to better-performing algorithms. The ensemble predictions identified eastern and southern Africa, including parts of Tanzania, Kenya, Mozambique, Zambia, Malawi, and Madagascar, as the regions of highest climatic suitability.

Moderate suitability was found in the West African savanna belt. Rainfall variables were the most important, especially rainfall in the wettest month (BIO13) and the driest month (BIO14).

Conclusions

The ensemble predictions indicate high climatic suitability for *C. cosyra* across large parts of East, Central and Southern Africa, and low suitability in hyper-arid and high-elevation regions. Uncertainty and model-agreement layers identify core high-confidence risk zones as well as environmentally marginal areas where predictions are less certain. Ongoing work will integrate land-use, host distribution, human-mediated pathways, genomic structure and future climate scenarios to refine habitat suitability estimates and support risk-based surveillance and area-wide pest management.

Session – Risk Assessment, Quarantine and Post-harvest

TruePest SWD. The Future of *Drosophila Suzukii*: Real-Time Monitoring System

Dhurgham Al Karawi, Shakir Al-Zaidi, Rachel Turner and James Pybis

Russell IPM LTD, 45 First Avenue, Flintshire, Industrial Park, CH5 2NU, United Kingdom. E-mail: dhurgham@russellipm.com

Background: Artificial intelligence (AI) is rapidly becoming an important driver of sustainable agriculture. By analysing vast datasets, AI provides actionable, accurate and real-time insights that support farmers and agribusinesses in better decision-making, resource optimisation and early detection of pests and diseases. SWD TruePest is an innovative AI-powered mobile application developed by Russell IOT LTD (part of the Russell IPM holding group) to streamline monitoring of the Spotted Wing Drosophila (*Drosophila suzukii*) in modern agriculture. The application enables soft fruit growers and field staff to detect and evaluate SWD populations in real time on farms. This advanced technology helps farmers shift from reactive, calendar-based interventions to a proactive, data-driven Integrated Pest Management strategy.

Methods: Using a phone camera, users scan specially designed sticky boards baited with kairomones specific to SWD. The App automatically identifies and counts SWD females and males, as well as non-target flies, especially vinegar flies (*Drosophila melanogaster*), within 30 seconds, without an internet connection. All data collected by this app is stored in a secure database, accessible via both the mobile app and a web portal.

Results: This innovation eliminates the time-consuming and error-prone manual counting process, providing real-time data on the SWD population with over 90% accuracy. The App also provides a colour-coded map to display high-risk areas on the farm and historical trends. This information helps farmers make decisions and apply targeted treatments in hotspot areas, thereby reducing unnecessary chemical use, lowering costs and minimising environmental impact. By comparing current readings with historical data, farmers can analyse and illustrate population trends over time and evaluate their control strategy.

Conclusion: SWD TruePest is a highly effective pest-monitoring application that minimises time-consuming tasks and human error, helping farmers make timely decisions for an effective IPM strategy, reducing fruit damage, and optimising production.

Keywords: *Drosophila suzukii*, SWD, real-time monitoring, integrated pest management, soft fruits, Artificial Intelligence

TEPHRIFADE: FAST AND HARMONISED DETECTION METHODS FOR QUARANTINE TEPHRITIDAE

Negin Ebrahimi¹, Wannes Dermauw¹, Annelies Kayenbergh², Tim Beliën³, Bart van de Vossenbergh⁴, Richard Gottsberger⁵, Eugénia de Andrade⁶, David Horta Lopes⁷, Norman Barr⁸, Terrance Todd⁸, Erin Campbell⁹, Matthias Becker¹⁰, Rebijith Balan¹¹, Hannes Schuler¹² & Marc De Meyer²

¹Flanders Research Institute for Agriculture, Fisheries and Food, Belgium; ²Royal Museum for Central Africa, Belgium; ³pcfruit Research Centre for Fruit Cultivation, Belgium; ⁴Netherlands Food and Consumer Products Safety Authority, The Netherlands; ⁵Austrian Agency for Health and Food Safety, Austria; ⁶The National Institute for Agrarian and Veterinary Research, Portugal; ⁷University of the Azores, Portugal; ⁸US Department of Agriculture, Animal and Plant Health Inspection Service, USA; ⁹Canadian Food Inspection Agency, Canada; ¹⁰Julius Kühn Institute, Germany; ¹¹Ministry for Primary Industries, New Zealand; ¹²Free University of Bozen-Bolzano, Italy. E-mail: negin.ebrahimi@ilvo.vlaanderen.be

Background: Tephritidae are major pests of fruit and vegetable crops worldwide due to their wide host range, high reproductive capacity and strong dispersal ability. In response to increasing introduction risks through global trade, the European Food Safety Authority (EFSA) has listed over 250 non-EU Tephritidae species as regulated quarantine pests, including four EU priority species. Rapid and reliable identification of these species is therefore essential for effective plant health protection in the European Union.

Methods: The Euphresco project TEPHRIFADE 1 conducted an inventory and evaluation of available diagnostic tools across European laboratories, including LAMP, qPCR, species specific PCR, multiplex PCR, CRISPR Cas12a, PCR RFLP and DNA barcoding approaches. Reference sequences were compiled from GenBank and BOLD databases to identify species lacking sequence data and to generate the missing reference sequences.

Results: TEPHRIFADE 1 demonstrated that rapid molecular methods are currently available for only a limited number of Tephritidae species. DNA barcoding was identified as the most used approach across laboratories. Challenges related to reproducibility and primer specificity were observed for some of the published methods, pointing out the need for thorough validation prior to routine laboratory implementation. Substantial gaps were detected in DNA databases, with 69 quarantine species lacking sequence data in GenBank and 80 species missing from BOLD. Despite efforts, the collection of specimens for these missing species proved challenging.

Conclusions: The gaps identified in TEPHRIFADE 1 highlight the need for improved and harmonised diagnostics for quarantine Tephritidae. In response, the TEPHRIFADE 2 project, starting in June 2026, will strengthen EU diagnostic capacity through the development of rapid molecular assays (qPCR or LAMP) and MALDI TOF mass spectrometry for *Anastrepha fraterculus* and *A. ludens*. The project will also standardise DNA extraction protocols, including rapid methods compatible with morphological identification, and address reference database gaps using institutional and museum collections, thereby enhancing EU preparedness for future incursions under increasing trade and changing climatic conditions.

Key words: fruit flies, molecular diagnostics, barcoding, LAMP, qPCR

KNOCK, KNOCK, KNOCKING ON EUROPE'S DOOR: REPEATED *DACUS* (DIPTERA: TEPHRITIDAE) INCURSIONS IN THE EUROPEAN UNION

Andrea Taddei^{1,3}, Sarah Chérasse¹, Raphaëlle Mouttet¹, Tefkros Iacovides², Despina Philippou²

¹ European Union Reference Laboratory (EURL) for Insects and Mites – ANSES
Laboratoire de la Santé des Végétaux - Unité Entomologie et botanique, 755 avenue du
campus Agropolis, CS 30016, FR-34988 Montferrier-sur-Lez Cedex, France

² Plant Protection Section, Department of Agriculture, Ministry of Agriculture,
Agricultural Development and Environment, 1412, Nicosia, Cyprus

³ andrea.taddei@anses.fr

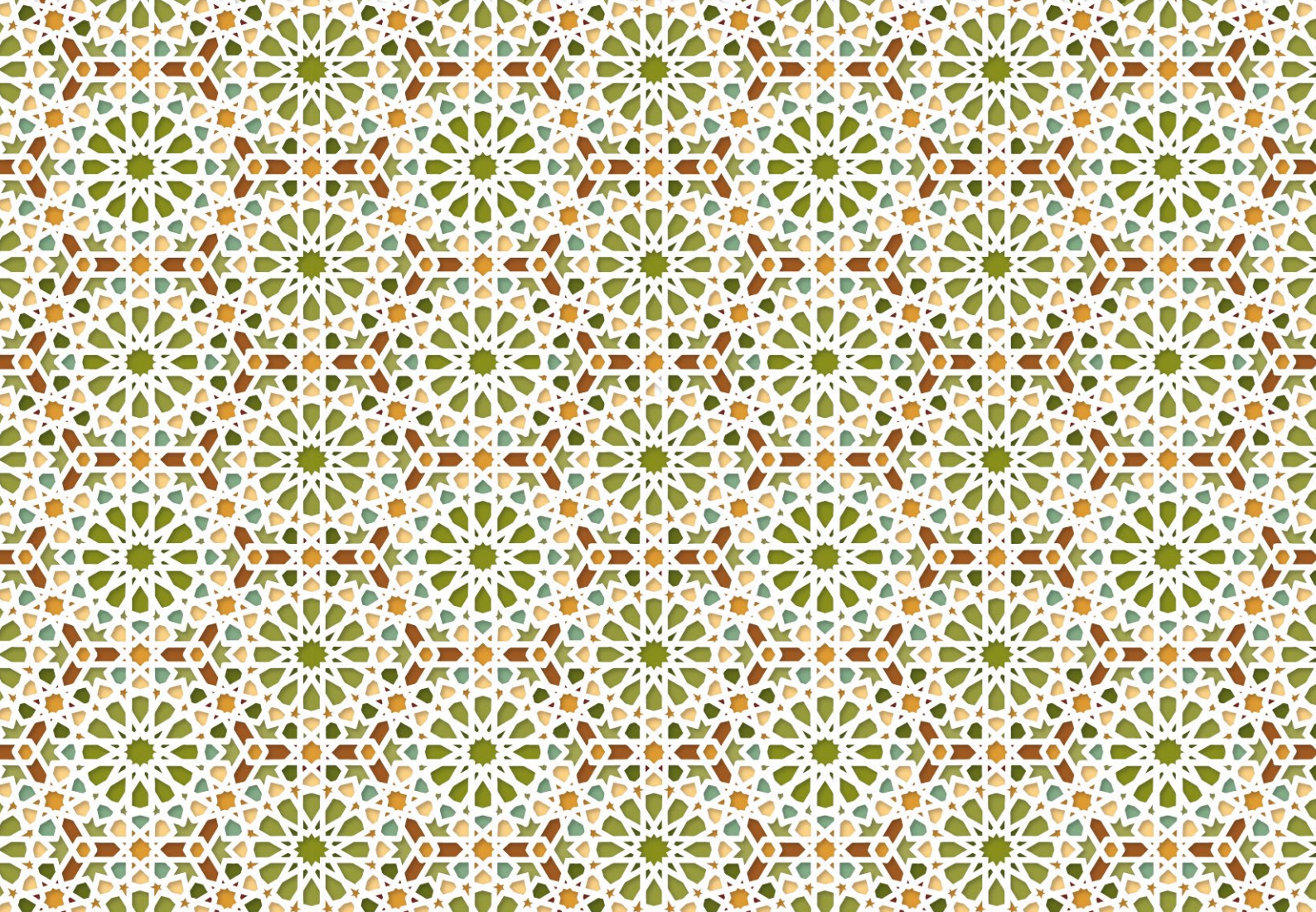
In Europe, *Dacus* fruit flies (Diptera: Tephritidae) represent an emerging phytosanitary threat. All species of this genus are listed as quarantine pests in the European Union meaning their introduction and spread should be prevented. *Dacus* spp. mostly originate in sub-Saharan Africa and attack a broad range of economically important cucurbits. Two species of agricultural significance, *Dacus ciliatus* Loew, 1862 and *D. frontalis* Becker, 1922,

In June 2024, *Dacus ciliatus* larvae were found for the first time in Cyprus in cucumber (*Cucumis sativus*). Samples were sent to the European Union Reference Laboratory (EURL) for insects and mites to confirm the identification with morphological and molecular methods. Since then, further inspections have detected larvae of this species in the fruits of cucurbit crops across several districts of the island. Phytosanitary measures were taken to attempt eradication. The same year, in July, two male *D. ciliatus* adults were captured in Palma Campania, Italy, in baited traps located near imported pumpkin consignments. No further incursions have been reported since.

In September 2024, a single *Dacus frontalis* adult specimen was intercepted in Brussels, Belgium, in a monitoring trap set near fruit and vegetable importers, representing the first incursion of this species in Europe. Follow-up surveillance found no further specimens. In September 2025, dipteran larvae were collected from a zucchini (*Cucurbita pepo*) fruit in a production site in Cannes, France. Morphological examination identified the larvae as belonging to the genus *Dacus*, while COI barcoding analysis suggested that the species was most likely *D. frontalis*. Baited traps were installed to catch adult specimens in order to confirm the species identity. Three male fruit flies were trapped in the same field where the infested zucchini fruits were found. Morphological examination confirmed the species as *Dacus frontalis*. This represents the first report of the species in mainland France. Additional sampling within the affected farm revealed the presence of more infested fruits.

These incursions highlight the risk of introduction and establishment of exotic fruit fly species in Europe. Coordinated surveillance, rapid and reliable identification, and phytosanitary measures are essential to prevent establishment and mitigate potential impacts on European horticulture.

Keywords: fruit fly, quarantine pest, *Dacus ciliatus*, *Dacus frontalis*, incursion, surveillance, Europe



POSTERS

Sterile Insect Technique

EFFECT OF THERMAL STRESS ON MATING COMPETITIVENESS IN *DROSOPHILA SUZUKII* FROM ARGENTINA

Alejandra C. Scannapieco^{1,2}, Romina M. Russo^{1,2}, Guillermo E. Bachmann¹,
Macarena Muniente¹, Fabián H. Milla¹; Diego F. Segura^{1,3}, María Clara Liendo¹
& Pablo Sambucetti⁴

¹Instituto de Genética, Instituto Nacional de Tecnología Agropecuaria (INTA), gv al Instituto de Agrobiotecnología y Biología Molecular (IABIMO) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Hurlingham, Buenos Aires, Argentina; ²Departamento de Producción Animal, Facultad de Agronomía, Universidad de Buenos Aires; Buenos Aires, Argentina; ³ Instituto de Investigación en Ciencias Agrarias y Veterinarias, Universidad del Salvador, Argentina; ⁴Instituto de Ecología, Genética y Evolución – CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. E-mail: scannapieco.a@inta.gob.ar

Background: *Drosophila suzukii* is a highly invasive pest causing severe economic losses in soft fruit crops worldwide, with a rapid expansion in Argentina. Its establishment across contrasting climatic regions suggests local thermal adaptation. However, how such adaptation influences reproductive performance under thermal stress remains unclear. Mating competitiveness is a determinant of population growth and a critical factor for the sterile insect technique (SIT), which depends on the ability of released males to successfully compete with wild males under field thermal conditions. Here, we assessed male mating competitiveness under thermal stress and evaluated the effect of heat hardening in two thermally differentiated populations of *D. suzukii*.

Methods: Mating pairs were established when flies were four days old. Mating assays were first conducted under three thermal conditions: 25°C, 28°C, and 31°C. Mating occurrence, latency to mate and mating duration were recorded for each pair. Two Argentine populations were evaluated: a warm-adapted population (Concordia) and a cold-adapted population (Río Negro). Then, we evaluated the effect of heat hardening on mating performance. The same variables were measured under three treatments: (1) mating at 25°C, (2) mating at 31°C without heat hardening, and (3) mating at 31°C following a heat-hardening pre-treatment (flies were exposed to 30°C for 1 h the day before the mating test).

Results: Mating frequency was similar at 25°C and 28°C, but significantly lower at 31°C in both populations. Mating latency did not differ among treatments. Temperature significantly affected mating duration, which was shorter at 31°C in both populations. At 28°C, mating duration was only reduced in the Río Negro population. Heat hardening did not modify the effect of high temperatures on any mating parameter.

Conclusions: Our results indicate that high temperatures negatively affect mating performance in *D. suzukii*, reducing mating frequency and duration. The reduced sensitivity of the warm-adapted population at intermediate temperatures supports the presence of population-specific thermal adaptation. The applied thermal pre-treatment was insufficient to mitigate the reproductive costs of high temperatures, with direct implications for population dynamics and SIT-based control strategies.

Key words: mating, spotted-wing *Drosophila*, heat-stress, thermal adaptation.

MEDITERRANEAN FRUIT FLY CONTROL STRATEGIES IN MOROCCO: TECHNICAL IMPLEMENTATION AND PERFORMANCE OF THE STERILE INSECT TECHNIQUE PROGRAM

Amine Leghlid, Yahya Machal, Rayhanna Adnasse & Hassan Zouhry
Sterile Insect Unit, Maroc Citrus, Agadir, Morocco
E-mail: Amineleghlid2@gmail.com

Background

The Mediterranean fruit fly (*Ceratitis capitata*) is a major pest of citrus worldwide, causing economic losses and export restrictions. Sustainable, environmentally friendly control strategies are essential. The Sterile Insect Technique (SIT), applied within an Area-Wide Integrated Pest Management (AW-IPM) framework, offers targeted suppression of medfly populations while reducing chemical use.

Methods

The Moroccan SIT program integrates large-scale sterile male production (up to 130 million/week capacity), Co-60 irradiation, aerial and ground releases, GIS-based release planning, trap network for monitoring, and laboratory quality control based on FAO/IAEA standards. In parallel, semi-field and laboratory evaluations assessed mating competitiveness, adult quality, egg-to-pupae conversion rate, and GSS (Vienna-8 fdf New Hybrid) strain stability. In this study, the performance of the field release of sterile flies is presented for the year 2025

Results

Before the start of SIT interventions, the medfly population was approximately 1 fly/trap/day (FTD). However, following the initiation of sterile male releases with an average of 2000 sterile males/week/ha, FTD values decreased to approximately 0.8~0.5 FTD after 52 weeks of treatment. Field release, trapping, and monitoring activities confirmed effective dispersal and distribution of sterile males across operational blocks.

Conclusion

The Moroccan SIT program demonstrates technical feasibility and scalability for sustainable medfly suppression. Integration of mass-rearing, adaptive releases, and quality control supports reduced pesticide use, improved fruit quality, and strengthened phytosanitary compliance. Full, sustained suppression of medflies requires ongoing rigorous implementation of the SIT program.

Keywords: *Ceratitis capitata*, Sterile Insect Technique, AW-IPM, citrus, Morocco, mass-rearing, pest suppression

**PATHOGENIC MICROORGANISMS ASSOCIATED WITH MASS REARING OF
ANASTREPHA FRATERCULUS (DIPTERA: TEPHRITIDAE)**

Ana L. Nussenbaum^{1,2}, Martina Miño¹, Micaela S. Garbalena¹, Beatriz Turinetti³,

Kostas Bourtzis⁴ & Diego F. Segura^{1,2}

¹ Instituto de Genética (INTA) – GV IABIMO (CONICET), Centro de Investigaciones en Ciencias Agronómicas y Veterinarias, Instituto Nacional de Tecnología Agropecuaria, T686, Hurlingham, Argentina. mail: nussenbaum.ana@inta.gob.ar

² Instituto de Investigación en Ciencias Agrarias y Veterinarias, Universidad del Salvador, Argentina.

³ ISCAMEN "Instituto de Sanidad y Calidad Agropecuaria de Mendoza", Mendoza, Argentina. ⁴ Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Austria.

Background: *Anastrepha fraterculus* is a major pest of fruit with significant economic impact in Argentina. The sterile insect technique (SIT), proposed for its control, requires mass rearing under artificial conditions. Such conditions may favor the proliferation of pathogenic bacteria that can compromise the quality of the released insects. This study aimed to isolate, identify, and characterize cultivable bacteria associated with different developmental stages of *A. fraterculus* and components of the rearing environment, as well as to assess their pathogenic potential.

Methods: Samples were aseptically collected from eggs, larvae, adults, larval diet, and water taken from the drinking containers of adult cages used in the rearing facility. Serial dilutions were plated on LB and MacConkey agar. Bacterial colonies were selected based on morphological characteristics, followed by biochemical tests (lactose fermentation and phenylalanine deamination). Molecular identification was performed by amplification and sequencing of the 16S rRNA gene, with sequences compared against reference databases (GenBank, SILVA). Selected strains were evaluated in pathogenicity bioassays by inoculating the artificial larval diet with 1x10⁷ CFU/mL. For each strain, 100 eggs were used per replicate (10 replicates per strain), along with a control treated with 0.9% NaCl. The number of pupae, the pupal weight, and the adult emergence were recorded.

Results: A high diversity of mesophilic bacteria and enterobacteria was detected, including the genera *Providencia*, *Morganella*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Aeromonas*, with genus composition varying among origins. In bioassays, one strain of the genera *Morganella* significantly reduced pupation, pupal weight, and adult emergence, while the others had no effect.

Conclusions: Mass rearing of *A. fraterculus* promotes the proliferation of opportunistic bacteria with pathogenic potential. The identification and monitoring of these microorganisms are essential to preserve the stability of the colonies and to ensure the biological quality of the reared insects. This study provides valuable information for improving biosecurity and microbiological management in SIT programs, contributing to the effectiveness of pest control strategies.

Key words: *Anastrepha fraterculus*, South American fruit fly, pathogens, mass rearing

EFFECT OF GUAVA ESSENTIAL OIL ON SEXUAL CALLING AND MATING IN *ANASTREPHA* SPP.

Guillermo E. Bachmann^{1, 2}, Francisco Devescovi^{1, 2}, Ana L. Nussenbaum^{1,2,3}, Micaela S. Garbalena^{1, 2}, Teresa M. Vera⁴ & **Diego F. Segura**^{1,2,3}

¹ Instituto de Genética, CICVyA, INTA, Argentina. mail: garbalena.micaela@inta.gob.ar

² CONICET, Argentina

³ Instituto de Investigación en Ciencias Agrarias y Veterinarias, USAL, Argentina

⁴ Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, IAEA, Austria
*bachmann.guillermo@inta.gob.ar

Abstract

Background: The genus *Anastrepha* includes species of economic importance because their larvae develop inside fruits of high commercial value. The sterile insect technique is an environmentally friendly control method that consists of releasing sterile males into crops so that they compete with wild males for females. The effectiveness of this technique depends on the ability of sterile males to court and mate with wild females. One way to enhance this ability is through aromatherapy with natural sources.

Methods: We evaluated the impact of guava essential oil (GEO) on the sexual behaviour of the Peruvian and Brazilian-1 morphotypes of *A. fraterculus*, as well as *A. obliqua* and *A. ludens*. Experiments were conducted in field cages under controlled conditions, assessing sexual calling frequency and mating success. Males were exposed to 20 µl of GEO from day 3 to day 12 post-emergence and were subsequently released into the experimental cages. In the first experiment, to evaluate sexual calling, groups of 8 exposed and 8 unexposed males, distinguished by colour marks, were introduced in the cages and the number of calling individuals was recorded. In the second experiment, 8 males from each treatment were released together with 8 females, and the number of copulations achieved by each male type was recorded.

Results: Results from the first experiment showed that males of both *A. fraterculus* morphotypes and *A. obliqua* significantly increased their calling frequency after GEO exposure, whereas *A. ludens* did not show significant differences. In the second experiment, GEO-exposed males achieved more copulations than unexposed males in the Brazilian morphotype of *A. fraterculus* and in *A. obliqua*.

Conclusions: Overall, the results indicate that exposure to GEO can enhance the copulatory success of males, not only in the Brazilian morphotype of *A. fraterculus*, but also in the Peruvian morphotype and in a related species such as *A. obliqua*.

Key words: sterile insect technique, aromatherapy, fruit flies, Tephritidae

Scaling Genetic Sexing Strains for Male-Only Releases to control *Ceratitidis capitata* Wied and *Anastrepha ludens* Loew.

Edwin Ramirez¹, Pedro Rendon², Miguel Lopez³, Cristobal Pezzarossi⁴, Corinna Bazelet⁵, Alene Seiler⁶, Jorge Lopez⁷, Yeudiel Gomez⁸,

Planta El Pino, Km 47.5, Parque Nacional Laguna El Pino, Barberena, Santa Rosa, Guatemala. E-mail: Edwin.ramirez@medfly.org.gt

Background: Fruit flies represent one of the most significant agricultural pests worldwide, affecting agricultural productivity, international trade, and food security. The Sterile Insect Technique (SIT) has become established as an effective and sustainable tool for the suppression of fruit fly (FF) pest populations. The use of Genetic Sexing Strains (GSS) has increased the effectiveness of SIT by enabling sex separation and the subsequent release of only males.

Methods: Two mass-rearing facilities in the Americas—the El Pino facility and the San Miguel Petapa facility of Guatemala’s Moscamed Program—implemented GSS for the mass production of sterile *C. capitata* and *A. ludens*, respectively. Implementation included the development and maintenance of genetically stable GSS, the rigorous application of rearing systems specifically designed for GSS, and the continuous improvement of rearing protocols. Key production control parameters monitored include larval density, diet quality, irradiation age, product (pupae/adult) quality control and the evaluation of sterile male field performance.

Results: We report here that the sustained use of GSS is technically and operationally viable at an industrial scale (1.2 billion males/week for *C. capitata* and 140 million sterile *A. ludens* per week). Both rearing facilities have supplied sterile males for the eradication (i.e. Dominican Republic) and subsequent maintenance of free areas and preventative release programs (i.e. continental U.S.; FL, CA, and TX) and constitute a key reference for other fruit fly control programs seeking to strengthen or implement SIT as a cornerstone of their integrated pest management strategies.

Conclusions: Experience in the mass rearing of *C. capitata* and *A. ludens* in both mass-rearing facilities has demonstrated that the success of GSS-based SIT does not rely solely on genetic components, but rather on an integrated strategy that includes process standardization, harmonization, and compliance with international quality standards, technical training, cost control, and regional cooperation.

Keywords: Genetic Sexing Strain, Sterile Insect Technique, Mass rearing

Operational monitoring of embryonic development in *Ceratitis capitata* eggs based on degree-hour accumulation in SIT mass-rearing facilities

Emmanuel Velázquez Dávila & José Antonio de la Cruz de la Cruz

Tapachula, Chiapas, Mexico, C.P. 30700 E-mail: emmanuel_vd@msn.com

Background: In sterile insect technique (SIT) mass-rearing facilities, egg hatch is a critical operational parameter, as it determines the effective transfer of biological material to subsequent production stages. Despite its importance, embryonic development of *Ceratitis capitata* eggs from oviposition to larval hatching is rarely monitored beyond hatch percentage, mainly due to the opacity of the chorion, which limits direct observation of internal structures. The lack of an operationally applicable method to monitor embryogenesis restricts early-stage quality control within production lines. This study aimed to establish an operational monitoring approach for embryonic development based on chorion clarification and degree-hour constant accumulation, enabling phase-by-phase identification of embryo development under mass-rearing conditions.

Methods: Eggs of *C. capitata* were obtained from a mass-rearing operation at the Moscamed facility in Metapa de Domínguez, Chiapas, Mexico. Egg collections were conducted at one-hour intervals between 07:00 and 14:00 h, obtaining an average of 200 eggs per day. Samples were incubated at a temperature of $25\pm 1^{\circ}\text{C}$ under high humidity. The last daily collection was designated as the reference point for oviposition time, and embryonic development was reconstructed using earlier collections. Observations were conducted at seven-hour intervals until larval hatching. Chorion clarification was performed using ethyl alcohol, allowing stereomicroscopic visualization and photographic documentation of internal embryonic structures. Degree-hour accumulation was calculated using a lower developmental threshold adopted from previously established thermal requirements for *C. capitata*.

Results: Embryonic development was operationally classified into five distinct stages. Stage 1 showed a homogeneous internal appearance with dispersed mitotically active cells. Stage 2 was characterized by blastoderm formation and increased internal organization. Stage 3 exhibited a distinct anterior protuberance corresponding to the future larval head. Stage 4 presented visible digestive structures, neural organization, and coordinated contraction movements. Stage 5 corresponded to a fully formed larva with clearly identifiable mouth hooks and posterior spiracles. These stages were consistently associated with approximately 95, 323, 475, 627, and 912 accumulated degree hours, respectively.

Conclusions: The use of ethyl alcohol as a chorion-clarifying agent enabled reliable visualization of embryonic development in *Ceratitis capitata* eggs under mass-rearing conditions. The classification of embryogenesis into five stages correlated with degree-hour accumulation provides a practical tool for early-stage quality control and biological monitoring in SIT facilities.

Key words: *Ceratitis capitata*, embryonic development, degree hours, egg hatch, sterile insect technique

Integrating sterile females into sterile insect technique programs to control *Bactrocera dorsalis* in La Réunion

Delatte Hélène¹, Amélie Lebon¹, Gabriel Jeanjeau¹, Laura Moquet¹

¹ CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France. Corresponding authors emails: helene.delatte@cirad.fr ; laura.moquet@cirad.fr

Background: The oriental fruit fly *Bactrocera dorsalis* is currently causing major economic losses in Réunion Island, threatening fruit production and agricultural sustainability. Conventional chemical control methods show important limitations, making environmentally friendly alternatives increasingly necessary. The Sterile Insect Technique (SIT), based on the mass release of sterile insects, has been identified as a promising biological control strategy. While SIT programs usually rely on the release of sterile males, the potential contribution of sterile females remains poorly documented, particularly regarding their influence on mating.

Methods: We assessed the effectiveness of SIT against *B. dorsalis* under Réunion Island conditions through a series of laboratory and semi-field experiments focusing on egg hatching rate, fecundity, and mating competitiveness. First lab experiments compared conditions with sterile males only, addition of both sterile males and sterile females, and addition of sterile females only. Egg hatch proportion and number of eggs laid per female were monitored over time. Then, mating trials were conducted in lab and semi-field cages to evaluate the preferences of wild and sterile females for wild versus sterile males (with also varying ratios).

Results: The introduction of sterile insects significantly reduced egg hatching rates, with the strongest effect observed in treatments including sterile males. The combined release of sterile males and females resulted in the lowest egg hatching proportions, suggesting a synergistic effect rather than a reduction in SIT efficiency. The presence of sterile females also led to a decrease in the total number of eggs laid, as expected due to their lack of fertility. Mating experiments revealed that wild females preferentially mated with sterile males when these were present in higher numbers, indicating satisfactory mating competitiveness despite a slight reluctance compared to wild males.

Conclusions: This first study provides some preliminary results on the potential effect of releasing sterile females on a SIT program against *B. dorsalis*. However, further investigations should be conducted. Future research should also focus on the potential impact of sterile female fruit-puncturing behavior before implementing large-scale field releases.

Key words: *Bactrocera dorsalis*, SIT, mating behaviour

COMPARISON OF TWO TYPES OF CELLULOSE BULKING AGENTS IN *CERATITIS CAPITATA* VIENNA 8 LARVAL DIETS

José Arredondo¹, Trinidad Artiaga-López¹, Nayeli Déctor¹, José Meza¹

Programa Moscamed (SADER-SENASICA), Metapa de Dominguez, Chiapas, 30860, Mexico. Email: jose.arredondo.i@senasica.gob.mx

Background: In solid larval diets, the bulking agent is the ingredient used in the second highest proportion after water. It is generally nutritionally inert and provide physical structure, volume, and consistency for larval movement and to maintain nutrient availability. It is a structurally fundamental component that determines the physicochemical microenvironment of the diet, the availability of nutrients, and directly influences insect yields and quality, as well as production costs and logistics. In this study, two diets with different cellulose bulking agent were evaluated for the larval development of *Ceratitis capitata* Vienna 8. One of them with CF-100 cellulose (TDS-Central Fiber CF-100, J Rettenmaier USA LP) and the other with RC-fine cellulose (ARBOCEL RC FINE, J Rettenmaier & söhn egmbh & co. kg).

Methods: Evaluations were conducted at the Moscamed México facility in the mother colony. The experimental unit consisted of five plastic trays with 5 kg of diet each. In each tray, 3 mL of eggs were deposited. Environmental conditions were maintained within a temperature range of 25 ± 1 °C and relative humidity of 85 to 90%. To the larval collections were used water-collection trays. Every 24 hours, and seven consecutive larval collections were performed. Larvae were recovered, dried with fine wheat bran to remove excess moisture, and placed in aluminum sieves for pupation. During the pupation period, environmental conditions were maintained at 24 ± 1 °C and 65 to 70% RH. The grams of pupae obtained per collection for each experimental unit were quantified, and the number of pupae collected was estimated. From this data, pupal yield (pupae/g of diet), the percentage of egg-to-pupa survival, the sex ratio based on puparium color, and pupal weight were estimated. Additionally, pupal samples were taken 48 hours prior to adult emergence to perform emergence and flight ability tests. Adult longevity without water or food was also estimated. For males, samples were taken from the first collection, and for females from the sixth. Adult fecundity and fertility were also measured.

Results: Analysis of the results indicated that pupal yields, percentages of egg-to-pupa survival, the female-male sex ratio, and the proportion of fliers showed no statistical differences between diets ($p > 0.05$). Significant differences ($p < 0.05$) were found in favor of RC-fine cellulose for pupal weights (males and females), adult emergence (females and males), female longevity without water or food, and adult fecundity.

Conclusions: These results demonstrate the effect of the bulking agent in the larval diet and the advantage of using RC Fine cellulose.

Key words: texturizing agent, quality, mass production, SIT

DEGREE-HOURS, A TOOL TO MEASURE THE MATURITY OF THE CERATITIS CAPITATA PUPA AND SYNCHRONIZE EMERGENCE IN MASS REARING SIT

José Antonio de la Cruz de la Cruz & Emmanuel Velázquez Dávila

Dirección postal en Tapachula, Chiapas, México, joseantonio.197931@gmail.com,
emmanuel_vd@msn.com

Background: *Ceratitis capitata* larval batches for the Sterile Insect Technique (SIT) are composed of multiple collections made at different times of day. This operational dynamic generates differences in pupal physiological age, which can result in variability in post-irradiation quality parameters evaluated at the production plant and the packing and release center. Conventionally, pupal maturity synchronization before irradiation is performed by visually assessing the pupal imaginal discs, providing a guide for corrective adjustments before irradiation without considering the thermal history of the pupation and maturation processes. The objective of this study was to develop and evaluate a quantitative methodology to estimate degree-hour accumulation to reduce variability in pupal maturity at the time of irradiation.

Methods: A thermal monitoring network was implemented using data loggers to continuously record ambient temperature and the temperature of biological material from larval collection, through pupation and maturation, until the introduction of irradiation. Using a thermal threshold for development adopted from the literature (10°C), the accumulation of degree-hours was calculated from the third larval instar to adult emergence. This information was integrated with conventional eye color assessments performed by the quality control unit, allowing for the establishment of a management program by transferring biological material between thermally differentiated areas to control heat accumulation.

Results: Fifty percent adult emergence was achieved between 3600 and 3900 cumulative degree-hours. The application of this method allowed for the synchronization of pupal maturity, reducing variability in emergence time. Thermal monitoring revealed temperature increases associated with metamorphosis processes, as well as the formation of isotherms within the pupal platforms. Fans were installed for ventilation and to homogenize thermal conditions. The evaluated quality parameters, including emergence time and flight capacity, remained within established standards.

Conclusions: Quantifying the cumulative degree-hours during the development of the immature stages of *C. capitata* allows for the transformation of a qualitative synchronization process into a quantifiable and reproducible operational procedure. This methodology homogenizes pupal maturity before irradiation, improves insect quality, and constitutes a transferable tool for standardizing processes in mass rearing facilities using insect isolation technology (SIT).

Key words:

Ceratitis capitata, degree-hours, pupal synchronization, Sterile Insect Technique, mass rearing

First genetic sexing strains of *Anastrepha ludens* (Diptera: Tephritidae) based on an X-autosome translocation

José S. Meza, Brenda Torres-Huerta, Jorge Ibañez-Palacios, Paola Fuentes-Fernández, Víctor García-Martínez

Departamento de Genética, Programa Operativo de Moscas, SADER-SENASICA/IICA, Metapa de Dominguez, Chiapas, 30860, Mexico

Background: Prolonged domestication can reduce genetic diversity and mating performance of genetic sexing strains (GSS) used in the sterile insect technique (SIT), requiring periodic genetic refreshment. However, conventional Y-autosome systems [T(Y;A)] are constrained by recombination-associated instability, operational dependence on filter rearing system, and limited feasibility of refreshment because wild individuals lack the translocation/marker configuration for mass crossing. An X-linked system [T(X;A)] can mitigate these constraints by linking the selectable marker to the X chromosome in homozygous condition, reducing recombination and enabling more viable refreshment strategies. Here, we report on the development and evaluation of the first *A. ludens* GSS based on an X-autosome chromosomal translocation, using the recessive *black pupae* mutation (*bp*).

Methods: Translocations were induced by irradiating *bp/bp* pupae (-2 d, 30 Gy/⁶⁰Co;) and mass-crossing males with wild-type (WT) virgin females. F₁ females were crossed individually (1:1) with WT males to establish independent families, followed by enrichment and subfamily selection across generations. Candidate lines were identified by their segregation patterns and confirmed by directed crosses. Confirmed lines were maintained under the application of the generational genetic filter system to monitor stability. Fertility was screened by comparing maximum egg hatch among WT, *bp*, Tapachula-7 [T(Y;A)] and the TX GSS.

Results: Across 22 induction bioassays, assays 20-21 produced numerous independent families, but only assay 20 advanced to confirmation and yielded two potential TX GSS: TX-366 and TX-373. Both showed segregation consistent with stable X-linked translocations and viable homozygous configurations [T(XX;AA)]. TX-373 maintained the expected sexing pattern through F₃ with no detectable recombination, whereas TX-366 showed a single recombinant female from brown pupae (0.03%) and an atypical phenotype under evaluation. Significant differences in fertility (maximum egg hatch) were observed among strains ($F_{4,40} = 58.63$; $p < 0.0001$); WT showed the highest values ($94.0 \pm 3.12\%$) and Tapachula-7 the lowest ($71.6 \pm 2.92\%$), whereas TX-366 and TX-373 were intermediate but remained significantly higher than Tapachula-7 ($77.7 \pm 4.27\%$ and $78.6 \pm 4.33\%$, respectively). Current work focuses on evaluating biological attributes, fecundity and quality-control parameters, together with cytogenetic characterization of the induced rearrangements.

Conclusions: TX-366 and TX-373 are the first confirmed *A. ludens* GSS based on T(X;A), providing a practical alternative to T(Y;A) systems. TX-373 showed higher early genetic stability, and both TX lines achieved higher egg hatch than Tapachula-7. These X-linked lines are candidate alternatives with potential operational advantages, but require systematic evaluation before implementation in mass rearing.

Key words: genetic sexing, male-linked translocations, recombination, mass rearing

Development of a genetic sexing strain in *Anastrepha fraterculus* (Wied.) from Peru by introgression of a sex sorting system based on a black pupae mutation

M. Teresa Vera¹, Sohel Ahmad¹, Edgardo Ortiz Carpio², Henry Troya³, Guillermo Bachmann⁴, Beatriz Carrizo⁵, Patricia Fernández⁶, Kostas Bourtzis¹, Walther Enkerlin¹, Polychronis Rempoulakis¹, Diego Segura^{4,5} & Carlos Cáceres¹

¹Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Austria; ²SENASA, Peru; ³AGROCALIDAD, Ecuador; ⁴CONICET, Argentina, ⁵INTA, Argentina; ⁶FAUBA, Argentina. m.t.vera@iaea.org

Background: The advantages of male-only releases in sterile insect technique (SIT) programmes encourage the development of genetic sexing strains (GSS) in different Tephritidae species. The South American fruit fly, *Anastrepha fraterculus* (Wied.), is a key pest in South America and actions to implement the SIT have been initiated in several countries. The availability of a GSS from a colony from Vacaria, Brazil (GSS-89), allows the release of only males in this species. However, *A. fraterculus* is a complex of cryptic species and eight morphotypes have been proposed with high levels of mating incompatibility. In this study, we introgressed the components of the genetic sexing mechanism of GSS-89 into the Peru morphotype to develop a GSS that can be released in operational programmes in this country.

Methods: Introgression experiments involved GSS-89 and a laboratory colony from Piura, Peru. The first cross involved males from Piura and females from GSS-89. The F1 was interbred to get black pupae females which were subsequently used to cross with GSS-89 males that carry the Y and autosome translocation as well as with Piura males to again perform siblings' crossings to get new black pupae females with a sequential increase of the Peruvian genome. After several similar crosses and backcrosses, the GSS-Piura was generated. To test if this new GSS was compatible with flies from Piura, standard field cage mating compatibility tests were conducted. In addition, male-borne volatiles of the three strains were collected and analysed, and a geometric morphometry analysis was conducted by defining 18 landmarks in the wings.

Results: The new GSS contained $\approx 97.5\%$ of the Peruvian genome. The Index of Sexual Isolation (ISI) estimated in mating compatibility studies showed compatibility between the GSS-Piura and the colony from Piura ($ISI = -0.15 \pm 0.05$) and high levels of incompatibility between the GSS-89 and the GSS-Piura ($ISI = 0.59 \pm 0.04$). Male-borne volatiles of the GSS-Piura were chemically closer to that of Piura males compared to the GSS-89 males. The same was observed for the wings' shape.

Conclusions: The high level of mating compatibility, together with the chemical profile and wing-shape results, confirms that the new GSS-Piura is similar and sexually compatible with the wild population from Piura, which clearly indicates that the introgression scheme was successful. This new GSS can now be scaled up for mass rearing to assess its genetic stability and further field releases and pilot trials.

Key words: South American fruit fly, GSS, mating compatibility, male borne volatiles, geometric morphometry.

Genetically engineered strains of Queensland fruit fly exhibit strong field performance

Md Forhad Hossain^{1,2}, Mst Raosonara Khatun¹, Md. Jamil Hossain Biswas¹, Zac Wylde¹, Vivian Mendez¹, and Phillip W. Taylor^{1*}

¹Applied BioSciences, Macquarie University, Sydney, NSW, Australia. ²Insect Biotechnology Division, IFRB, AERE, Bangladesh Atomic Energy Commission, Bangladesh. ³School of Natural Sciences, Macquarie University, Sydney, NSW, Australia. ⁴South Australian Research and Development Institute, Adelaide, SA, Australia. E-mail: phil.taylor@mq.edu.au

Background: Queensland fruit fly (Q-fly) (*Bactrocera tryoni*), is one of the most significant horticultural pests in Australia. The sterile insect technique (SIT) is currently employed for regional management of Q-fly outbreaks and has strong potential to be used in endemic areas to suppress this pest population. Recently, genetically engineered strains have been developed intending to SIT efficiency including releasing only male flies [Genetic Sexing Strain (GSS) of Greyscale Exon-3 strain] and phenotypic marking for monitoring by yellow-coloured body (Yellow-1).

Methods: We performed mark-release-recapture trials to compare the field performance of these engineered strains with unengineered laboratory strain currently utilized in SIT programs. The present study assesses field recapture rate and dispersal distance of these engineered strains along with other standard quality control parameters such as adult emergence rate and flight ability. To evaluate the effect of dye, Yellow-1 strain were released in both dyed and undyed forms.

Results: Undyed Yellow-1 males had significantly higher recapture rates and greater dispersal distance than all other strains. GSS had comparable performance to that of standard laboratory flies. Adult emergence and flight ability were comparable in both of the tested strains with the current laboratory strains. Dyed Yellow-1 males showed reduced recapture rate and dispersal compared to undyed Yellow-1 males, demonstrating a significant effect of marking.

Conclusions: The field performance of both of the Yellow-1 and GSS strains were satisfactory for SIT programs, and undyed Yellow-1 males even outperformed unengineered laboratory males. These findings indicate suitability of these strains, which is an important milestone for reducing costs, and enhancing operational safety and efficiency of operational SIT program.

Key words: *Bactrocera tryoni*, sterile insect technique, Genetic sexing strain, field-release-recapture

Semi-field experiments comparing wild and laboratory-reared sterile males of Mediterranean fruit flies

Yahya Machal, Amine Leghlid, Rayhanna Adnasse & Hassan Zouhry

Sterile Insect Unit, Maroc Citrus, Agadir, Morocco

E-mail: yahyamachal@gmail.com

Introduction

The Mediterranean fruit fly (*Ceratitidis capitata*) is a major citrus pest causing economic losses and limiting exports. The Sterile Insect Technique (SIT), applied in an Area-Wide Integrated Pest Management (AW-IPM) framework, provides a sustainable, chemical-reducing strategy for population suppression.

Methods

The Moroccan SIT program combines large-scale sterile male production (up to 130 million/week), Co-60 irradiation, aerial and ground releases, and trap-based monitoring under FAO/IAEA standards. In this study, semi-field and laboratory tests assessed mating competitiveness, adult quality, egg-to-pupae conversion, and the stability of the Vienna-8 FDF New Hybrid (GSS) strain, as well as the impact of the selected irradiation dose on the male fitness .

Results

Semi-field experiments comparing wild and laboratory-reared males demonstrated satisfactory **Index of Sexual Competitiveness (ISR 0.46) and of Relative Isolation Index (RII 2.3)**. Mass-rearing and laboratory quality control assessments demonstrated stable and high biological performance of the strain production . Egg quality tests showed approximately **80% hatch without incubation, 15% hatch at seeding, and 85% hatch after incubation**. Egg-to-pupae conversion efficiency of the new hybrid strain reached approximately **50% in the filter colony using sugar beet pellets-based larval diet, and 43% in the filter colony using wheat bran-based larval diet**, indicating improved larval development and recovery rates depending on diet formulation. Pupal quality parameters indicated an average pupal weight of **8.2 mg**. quality assessments showed **90% emergence rate, 84% flight ability, and 78% survival under stress conditions**. Genetic stability monitoring indicated a recombinant rate below **1.8%**, and the fertility levels of released males remained low at approximately **1.8% residual fertility**, confirming the adequate irradiation dose.

Conclusions

The Moroccan SIT program is feasible and scalable. Continuous implementation is required for sustained suppression, reduced pesticide use, and improved citrus quality. *Keywords: Ceratitidis capitata*, Sterile Insect Technique, AW-IPM, citrus, Morocco, mass-rearing, pest suppression

Effects of irradiated female *Ceratitis capitata* (V-8 strain) on Nadorcott mandarin fruit quality under laboratory conditions

Said Aminzou¹, Karim Ahmed Said¹, Amine Leghlid², Toufik Salhi³ & Abdelaziz Zahidi^{1,4}

¹: Laboratory of Applied Entomology, Agadir Horticultural Complex, IAV Hassan II, Agadir, Morocco

²: Sterile Male Production Center, Ait Melloul, Morocco

³: Nadorcott Variety Producers Association (APMN), Morocco

⁴: Laboratory of Biotechnology and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco

E-mail : s.aminzou@cha-agadir.ma

Background: The Mediterranean fruit fly (*Ceratitis capitata*) is a key pest of citrus orchards in Morocco. To reduce dependence on chemical insecticides, the Sterile Insect Technique (SIT) has been adopted as an environmentally sustainable control strategy based on the mass-rearing, irradiation, and release of sterile males. However, the accidental release of sterile females may occur and raise concerns regarding fruit damage caused by oviposition attempts. This study aimed to evaluate the biological performance and oviposition behavior of irradiated female *Ceratitis capitata* and their potential impact on Nadorcott mandarin fruit quality under laboratory conditions.

Methods: Female pupae of the V-8 strain were irradiated at three pre-emergence intervals of 24, 48 and 72 hours prior to adult emergence. Emergence rate, flight ability, adult longevity, and oviposition punctures on healthy fruits were assessed and compared with non-irradiated female controls.

Results: Irradiation applied 24 hours prior to emergence resulted in the lowest biological performance, with emergence, flight ability, and survival rates of 64%, 61%, and 65%, respectively. In contrast, females irradiated at 72 hours before emergence exhibited significantly higher performance, with emergence, flight ability, and survival rates reaching 83%, 72%, and 80%, respectively, values close to those observed in non-irradiated controls. Non-irradiated females showed the highest oviposition activity, averaging approximately 6.5 punctures per fruit per day, whereas irradiated females induced fewer punctures, particularly those treated at 24 hours. Nevertheless, despite being sterile, irradiated females retained oviposition behavior sufficient to negatively affect the commercial quality of citrus fruits.

Conclusion: The results confirm that irradiated female *Ceratitis capitata*, although sterile, can maintain oviposition activity leading to potential fruit quality depreciation. Strengthening genetic sexing systems and optimizing irradiation timing are therefore essential to minimize female release and mitigate unwanted fruit damage in operational SIT programs.

Key words: *Ceratitis capitata*, Sterile Insect Technique, irradiation timing, quality control, oviposition behavior, citrus, Nadorcott.

**EFFECT OF DIFFERENT IRRADIATION DOSES ON CERTAIN BIOLOGICAL
PARAMETERS OF THE FRUIT FLY *BACTROCERA DORSALIS* HENDEL (DIPTERA:
TEPHRITIDAE) UNDER LABORATORY CONDITIONS**

Delphine H. Zaoua^{1*}, Karim Nébié², Remy A. Dabiré¹, Issaka Zida², Fernand Sankara¹

¹Université Nazi Boni, Institut de Développement Rural

²Centre National de la Recherche Scientifique et Technologique, Institut de l'Environnement et de Recherches Agricoles

*Corresponding author: delphinezaoua@gmail.com

Background: The fruit fly *Bactrocera dorsalis* causes significant damage and hinders the development of the mango industry in Burkina Faso. Faced with this biotic constraint, it is necessary to develop sustainable control methods, including the sterile insect technique. This study aimed to determine an optimal irradiation dose to sterilize male of *B. dorsalis* in order to control the pest.

Methodology: The effect of three irradiation doses (60 Gy, 65 Gy, 70 Gy) was evaluated and compared with control on pupal hatching rate, fertility, sex ratio, survival, and flight ability. To this end, each dose was applied to four batches of 200 six-day-old pupae placed in Petri dishes.

Results: The pupal hatching rate was not influenced by the radiation dose ($P > 0.05$). It ranged from 91% (60 Gy) to 93.75% (70 Gy). The sex ratio was 0.88 in the untreated control and from 1.03 to 1.14 in the irradiated individuals. The hatching rate of eggs laid by unirradiated females mated with unirradiated males was 78.3%. In contrast, in unirradiated females mated with irradiated males, the egg hatching rate ranged from 0% (70 Gy) to 4.46% (60 Gy). At a dose of 70 Gy, the oviposition capacity of irradiated females was inhibited. After 18 days of monitoring the emerged flies, 100% of the unirradiated males were still alive in the control treatment. In contrast, the survival rate of the irradiated males ranged from 67.5% (60 Gy) to 77.50% (70 Gy). The percentage of flies able to fly ranged from 86.06% (65 Gy) to 93.89% (60 Gy), with a predominance of males. No significant difference ($P = 0.296$) was observed between the treatments for this parameter.

Conclusions: The irradiation dose of 70 Gy renders male *B. dorsalis* completely sterile while maintaining good flight capacity and an acceptable male survival rate. Further studies in semi-field environments are needed for validation.

Key words: *Bactrocera dorsalis*, irradiation, biological parameters, biological control.

Scaling Genetic Sexing Strains for Male-Only Releases to control *Ceratitis capitata* Wied and *Anastrepha ludens* Loew.

Edwin Ramirez¹, Pedro Rendon², Miguel Lopez³, Cristobal Pezzarossi⁴, Corinna Bazelet⁵,
Alene Seiler⁶, Jorge Lopez⁷, Yeudiel Gomez⁸,

Planta El Pino, Km 47.5, Parque Nacional Laguna El Pino, Barberena, Santa Rosa,
Guatemala. E-mail: Edwin.ramirez@medfly.org.gt

Background: Fruit flies represent one of the most significant agricultural pests worldwide, affecting agricultural productivity, international trade, and food security. The Sterile Insect Technique (SIT) has become established as an effective and sustainable tool for the suppression of fruit fly (FF) pest populations. The use of Genetic Sexing Strains (GSS) has increased the effectiveness of SIT by enabling sex separation and the subsequent release of only males.

Methods: Two mass-rearing facilities in the Americas—the El Pino facility and the San Miguel Petapa facility of Guatemala’s Moscamed Program—implemented GSS for the mass production of sterile *C. capitata* and *A. ludens*, respectively. Implementation included the development and maintenance of genetically stable GSS, the rigorous application of rearing systems specifically designed for GSS, and the continuous improvement of rearing protocols. Key production control parameters monitored include larval density, diet quality, irradiation age, product (pupae/adult) quality control and the evaluation of sterile male field performance.

Results: We report here that the sustained use of GSS is technically and operationally viable at an industrial scale (1.2 billion males/week for *C. capitata* and 140 million sterile *A. ludens* per week). Both rearing facilities have supplied sterile males for the eradication (i.e. Dominican Republic) and subsequent maintenance of free areas and preventative release programs (i.e. continental U.S.; FL, CA, and TX) and constitute a key reference for other fruit fly control programs seeking to strengthen or implement SIT as a cornerstone of their integrated pest management strategies.

Conclusions: Experience in the mass rearing of *C. capitata* and *A. ludens* in both mass-rearing facilities has demonstrated that the success of GSS-based SIT does not rely solely on genetic components, but rather on an integrated strategy that includes process standardization, harmonization, and compliance with international quality standards, technical training, cost control, and regional cooperation.

Keywords: Genetic Sexing Strain, Sterile Insect Technique, Mass rearing

Optimum irradiation dose for sterilizing adult male *Bactrocera dorsalis* Hendel

Enoch Selorm Kofi Ofori¹, Benjamin Offei¹, Peter Davor¹, Stanley Acquah¹, Francis Apaatah¹, Michael Yao Osae¹, Peter Kofi Kwabong² & John Abraham²

¹Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P. O. Box LG 80, Legon, Accra, Ghana.

²Department of Conservation Biology and Entomology, School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, PMB, Cape Coast, Ghana. E-mail: enoch.ofori@gaec.gov.gh

Background: The Sterile Insect Technique (SIT) is an environmentally friendly and species-specific method for managing tephritid fruit flies, but its large-scale application is often constrained by the high costs of mass rearing. Trap-Irradiate-Release (TIR-SIT), which relies on sterilizing wild-caught males, offers a cost-effective alternative. However, successful implementation depends on identifying an irradiation dose that induces high sterility while preserving male reproductive performance. This study determined the optimum gamma irradiation dose for sterilizing adult male *Bactrocera dorsalis* and evaluated its effects on female fecundity and egg hatchability.

Methods: Wild adult male *B. dorsalis* were collected from mango orchards in eastern Ghana using methyl eugenol-baited traps and acclimatized under controlled insectary conditions. Groups of males were irradiated with gamma doses of 0 (control), 10–100 Gy using a Cobalt-60 source. Irradiated males were mated with laboratory-reared virgin females, after which female fecundity and egg hatchability were assessed. Hatchability data were analyzed using ANOVA and log-dose probit analysis to estimate the dose inducing 95% sterility (LD₉₅).

Results: Egg hatchability declined significantly with increasing irradiation dose ($p < 0.001$), showing a strong negative dose–response relationship ($r^2 = 0.903$). Probit analysis estimated an LD₉₅ of 72.49 Gy (95% CI: 56.4–106.4 Gy), with 70 Gy resulting in approximately 95% sterility. Hatchability at doses ≥ 70 Gy was $\leq 3\%$, compared with 81% in the control. In contrast, female fecundity was not significantly affected by mating with irradiated males across all tested doses ($p > 0.05$), indicating that irradiation impaired male fertility without suppressing female oviposition.

Conclusions: An irradiation dose of 70 Gy effectively induces near-complete sterility in adult male *B. dorsalis* without adversely affecting female fecundity, representing an optimal balance between reproductive sterility and biological performance. These findings support the feasibility of adult-male irradiation for TIR-SIT and provide a critical operational parameter for developing cost-effective, area-wide management programs for *B. dorsalis*, particularly in resource-limited settings.

Key words: Irradiation, Trap-Irradiate-Release, Sterile Insect Technique, *Bactrocera dorsalis*, Fecundity

Longevity, dispersal, and performance of *Drosophila suzukii* sterile males in cherry

Ghais Zriki, Lisa Haddad, Jugurtha Ouzzir, Christine Fournier, Capucine Perrey, Sarah Quemini, Jeanne Monnier & David Monnin

Interprofessional Technical Centre for Fruit and Vegetables (CTIFL), 751 le Balandran, 30127 Bellegarde, France. E-mail: ghais.zriki@ctifl.fr

Background: *Drosophila suzukii* (Spotted-Wing Drosophila, SWD) is a major pest of small berries and soft-skinned fruits worldwide. Effective management of SWD is costly and relies mainly on chemical insecticides and labor-intensive cultural practices. The Sterile Insect Technique (SIT) is a species-specific, environmentally friendly, and cost-effective method successfully used against several major insect pests, including fruit flies. Sterile Insect Technique has also been shown to suppress SWD in strawberry and raspberry. Cherry production, a high-value crop, is severely affected by SWD. Integrating SIT into SWD management strategies in commercial cherry orchards is therefore promising. Successful implementation of SIT depends on the performance of sterile males, which requires evaluation of key parameters under field conditions.

Methods: The performance of SWD sterile males was evaluated in field cages and in netted cherry orchards during the 2025 growing season. Studied parameters included survival, longevity, dispersal, and reproductive competitiveness. Adult males of SWD were sterilized by exposing mature pupae to X-ray radiation at a dose of 110 Gy. Unfed 24-h-old sterile males were released in cages containing a small potted cherry tree, and daily survival was evaluated. A mark–release–recapture (MRR) method was used to assess dispersal and longevity of sterile males in a fully net-enclosed cherry orchard. Reproductive competitiveness was evaluated by comparing the percentage of larva-infested cherries in control and sterile-release plots. Each plot contained four full-grown cherry trees covered with protective netting.

Results: In field cages, males irradiated at 110 Gy survived an average of 2.3 days, compared with 2.1 days for non-irradiated males. In netted cherry orchards, on average, sterile males dispersed up to 15 m in summer and 25 m in spring. Twice-weekly releases of sterile males reduced fruit infestation by 16% compared with controls.

Conclusions: These results indicate that 110 Gy X-ray irradiation produces sterile *D. suzukii* males with adequate quality and performance, supporting SIT as a promising component of integrated SWD management in cherry production.

Keywords: Fruit flies, Spotted-Wing Drosophila, reproductive competitiveness, Sterile Insect Technique

DEGREE-HOURS, A TOOL TO MEASURE THE MATURITY OF THE CERATITIS CAPITATA PUPA AND SYNCHRONIZE EMERGENCE IN MASS REARING SIT

José Antonio de la Cruz de la Cruz & Emmanuel Velázquez Dávila

Dirección postal en Tapachula, Chiapas, México, joseantonio.197931@gmail.com,
emmanuel_vd@msn.com

Background: *Ceratitis capitata* larval batches for the Sterile Insect Technique (SIT) are composed of multiple collections made at different times of day. This operational dynamic generates differences in pupal physiological age, which can result in variability in post-irradiation quality parameters evaluated at the production plant and the packing and release center. Conventionally, pupal maturity synchronization before irradiation is performed by visually assessing the pupal imaginal discs, providing a guide for corrective adjustments before irradiation without considering the thermal history of the pupation and maturation processes. The objective of this study was to develop and evaluate a quantitative methodology to estimate degree-hour accumulation to reduce variability in pupal maturity at the time of irradiation.

Methods: A thermal monitoring network was implemented using data loggers to continuously record ambient temperature and the temperature of biological material from larval collection, through pupation and maturation, until the introduction of irradiation. Using a thermal threshold for development adopted from the literature (10°C), the accumulation of degree-hours was calculated from the third larval instar to adult emergence. This information was integrated with conventional eye color assessments performed by the quality control unit, allowing for the establishment of a management program by transferring biological material between thermally differentiated areas to control heat accumulation.

Results: Fifty percent adult emergence was achieved between 3600 and 3900 cumulative degree-hours. The application of this method allowed for the synchronization of pupal maturity, reducing variability in emergence time. Thermal monitoring revealed temperature increases associated with metamorphosis processes, as well as the formation of isotherms within the pupal platforms. Fans were installed for ventilation and to homogenize thermal conditions. The evaluated quality parameters, including emergence time and flight capacity, remained within established standards.

Conclusions: Quantifying the cumulative degree-hours during the development of the immature stages of *C. capitata* allows for the transformation of a qualitative synchronization process into a quantifiable and reproducible operational procedure. This methodology homogenizes pupal maturity before irradiation, improves insect quality, and constitutes a transferable tool for standardizing processes in mass rearing facilities using insect isolation technology (SIT).

Key words:

Ceratitis capitata, degree-hours, pupal synchronization, Sterile Insect Technique, mass rearing

POPULATION DYNAMICS AND STERILE INSECT TECHNIQUE FOR IPM OF *DROSOPHILA SUZUKII* IN MOROCCO

Khalid Khfif¹, Hasnae Ennouinou^{1,2,3}, Khalid Fatouaki⁴, Mohammed Mouhib⁴

¹Laboratory of Entomology, Research Unit on Nuclear Techniques, Environment, and Quality, National Institute for Agricultural Research (INRA), 78 Bd. Sidi Med. Ben Abdellah, 90010 Tangier, Morocco

²Department of Plant Protection, National School of Agriculture of Meknes, Km10, Rte Haj Kaddour, BP S/40, Meknes, 50001, Morocco

³Plant Protection Unit, Hassan II Agronomic and Veterinary Institute, Rabat, Morocco

⁴Research Unit on Nuclear Techniques, Environment and Quality, National Institute of Agronomic Research (INRA), Tangier, Morocco

*Corresponding author: khalid.khfif@inra.ma

Background: *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), is an invasive pest causing substantial economic losses in soft fruit production worldwide. In Morocco, infestations in raspberry orchards are increasingly threatening the competitiveness of the export sector, especially under stricter regulations limiting chemical pesticide use.

Methods: To explore sustainable management options, this study combined field monitoring, laboratory bioassays, and sterile insect technique (SIT) feasibility trials under laboratory conditions. Population dynamics were monitored weekly in raspberry cultivars using banana-yeast baited traps until the end of May.

Results: Infestation levels were consistently higher in the Yazmin cultivar, which exhibited three annual peaks of *D. suzukii* adult flies, while a total of six generations were predicted based on accumulated degree-days. In parallel, SIT protocols were developed using cobalt-60 irradiation facility at INRA-Tangier, Morocco. Reciprocal crosses performed at irradiation doses of 180–210 Gy revealed that females exposed at all tested levels became completely sterile, whereas irradiated males retained partial fertility, particularly at intermediate doses. When irradiation was applied at the pupal stage, adult emergence was markedly reduced (37–45%), and males exhibited higher post-emergence mortality compared to females, indicating a sex-specific sensitivity to radiation. Laboratory assays confirmed the pathogenicity of isolate ‘L19’ fungal suspensions (10^6 – 10^8 conidia/mL) on wild with more sensitivity for irradiated *D. suzukii* populations.

Conclusions: This study provides the first integrated assessment of *D. suzukii* population dynamics in Moroccan raspberry systems and demonstrates the potential of biological control agents and SIT. These combined approaches offer promising components of an eco-friendly IPM strategy capable of reducing pesticide reliance and strengthening the resilience of Morocco’s berry export sector.

Key words: Degree-day modelling, Sterile insect technique, Entomopathogenic fungi, Integrated pest management.

BIOLOGICAL PERFORMANCE OF *ANASTREPHA FRATERCULUS* FOLLOWING ADULT INJECTION: IMPLICATIONS FOR SIT DEVELOPMENT

M. Celeste Soria¹, Fabián H. Milla¹, M. Cecilia Giardini¹, Romina M. Russo¹, Diego F. Segura^{1,2}, Alejandra C. Scannapieco¹ & Silvia B. Lanzavecchia¹

¹ Instituto de Genética GV-IABIMO UEDD CONICET-INTA, Instituto Nacional de Tecnología Agropecuaria, Aristizabal and El Nandú, 1686 Hurlingham, Buenos Aires, Argentina. ² Instituto de Investigación en Ciencias Agrarias y Veterinarias, USAL, Argentina.

E-Mail: soria.mariaceleste@inta.gob.ar

Background: The South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae) is a major pest in South America and co-occurs with *Ceratitis capitata* in sub-tropical and temperate regions of Argentina. Both species are important quarantine pests. While the sterile insect technique (SIT) has been successfully implemented against *C. capitata*, its application to *A. fraterculus* is still under development. The generation of genetically sexed strains, potentially through CRISPR/Cas9-based gene editing, represents a critical step for effective SIT implementation. The DIPA-CRISPR approach, based on direct injection of gRNA/Cas9 complexes into adult insects, is a promising alternative; however, its success depends on optimizing injection protocols and assessing their impacts on key biological traits.

Methods: We evaluated biological performance and survival following mesothoracic injection in *A. fraterculus* adult females. Newly emerged females were injected with 2 µl of 0.9% NaCl solution using a Hamilton microsyringe and compared with non-manipulated and punctured-only controls. Fecundity was analyzed using generalized linear models, while fertility and survival were evaluated descriptively. In a second assay, we assessed injection accuracy using visual tracer dyes at 1.5%, 2%, and 16.6% (v/v) in 0.9% NaCl, with survival used as the response variable.

Results: Mesothoracic injection did not significantly affect oviposition probability ($\chi^2 = 1.5477$; $p = 0.4612$). However, injected females exhibited a 19.6% reduction in fecundity compared to non-manipulated controls ($\chi^2 = 34.676$; $p = 2.953 \times 10^{-8}$). Fertility remained high and comparable across treatments (0.96 ± 0.02 in controls, 0.85 ± 0.06 in punctured females, and 0.93 ± 0.01 in injected females). Survival exceeded 85% at 12 days post-treatment in all groups. In the tracer dye assay, survival ranged from 45% to 90% depending on dye concentration.

Conclusions: Mesothoracic injection in adults constitutes a reliable and minimally invasive technique that preserves survival and reproductive performance. These findings support its applicability in functional genomics and genetic control studies aimed at developing SIT-based strategies for *A. fraterculus*.

Key words: *Anastrepha fraterculus*, South American fruit fly, biological performance, Sterile Insect Technique (SIT), CRISPR/Cas9

Evaluation of New X-Chromosome Translocation Genetic Sexing Strains of *Ceratitis capitata*.

Santiago Jacobo Aguirre Ibañez¹, Martha Nayeli Guillen Rivera¹, Christian Stauffer², Polychronis Rempoulakis¹ & Maria Teresa Vera¹.

¹Insect Pest Control Section, FAO/IAEA, Austria. E-mail:s.j.ibanez@iaea.org.

²Boku University, Vienna, Austria.

Background: The Vienna-8 genetic sexing strain (GSS), defined by a Y-autosome translocation T(Y;A), white pupae, and a temperature-sensitive lethal mutation (*tsI*), is widely mass-reared for sterile insect technique (SIT) applications against *Ceratitis capitata*. However, the large-scale production of this strain presents significant challenges, as females emerge later than males during the adult stage, complicating production logistics. In addition, this genetic configuration makes the introgression of wild material difficult, limiting the potential to improve the sexual performance of the strain. A GSS carrying an X-autosome translocation T(XX;AA) can overcome these constraints. First, they present a faster female development compared with Vienna-8. Second, they allow the introgression of wild genes in an easier and faster way, enabling timely improvement of the colony. The aim of this study was to characterize several T(XX;AA) to identify the strain with the highest potential for mass-rearing, through the assessment of standard quality control parameters across different strains.

Methods: Seven T(XX;AA) strains were evaluated: four with potentially different X-autosome breakpoints, one sharing the same breakpoint but belonging to different families, and two refreshed strains. Vienna-8 GSS served as the control. Key mass-rearing parameters were assessed: fecundity, fertility, egg-to-pupa and egg-to-adult recovery, adult emergence, sex ratio, flight ability, and male competitiveness. A thermal sensitivity test was also performed at temperatures ranging from 31 to 36 °C to establish the thermal sensitivity of males and females at the embryo stage. The genetic stability of each strain was monitored for more than 20 generations.

Results: Among the five non-refreshed T(XX;AA) translocation lines, one strain showed superior performance across most quality control parameters. In contrast, the incorporation of wild genetic material into TX-IPCL-3 led to a marked improvement in viability, quality, and genetic stability in its refreshed counterpart (TX-IPCL-3.1), although performance remained below that of Vienna-8. Thermal sensitivity differed between TX-IPCL-3 and TX-IPCL-3.1, shifting from 35 °C to 34 °C. Regarding genetic stability, one non-refreshed strain reached recombination levels above 10% from generation 18 onward, whereas its refreshed version remained below 2% for more than 20 generations, consistent with the remaining T(XX;AA) strains.

Conclusions: This study identified one GSS based on X-autosome translocation with strong potential for mass-rearing, particularly after a genetic refreshment. This significantly improved its viability, quality, thermal sensitivity, and long-term stability. While Vienna-8 remains the most robust strain overall, the performance of several evaluated lines suggests that new GSS candidates could be further optimized for future SIT applications.

Key Words: Genetic sexing strains (GSS), X-autosome translocation, Quality control.

Session 6. Sterile Insect Technique.

Sterilization dose with Gamma and X-ray irradiation in *Zeugodacus cucurbitae*

Savitree Raghoo¹, Jhonatan Sanchez-Aguilar², Vanessa S. Dias², Md. Al-arafat Topu², Hanano Yamada², Polychronis Rempoulakis² & M. Teresa Vera²

¹Entomology Division, Agricultural Services. Ministry of Agro-Industry, Food Security, Blue Economy and Fisheries, Reduit, Republic of Mauritius; ²Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, IAEA, Austria. Email: sraghoo@govmu.org

Background: The melon fruit fly, *Zeugodacus cucurbitae*, is one of the major pests affecting cucurbit crops in Mauritius. The current area-wide integrated pest management (AW-IPM) program in Mauritius includes sanitation campaigns, protein bait sprays, pest monitoring, and the release of sterile flies. From all applied measures of this AW-IPM program, the sterile insect technique (SIT) constitutes a critical component, as it is an environmentally friendly measure. The effectiveness of SIT depends on the production of high-quality sterile males capable of successfully competing with wild males for females in the field. Optimising the irradiation dose for sterilization is essential to minimize somatic damage and maintain male quality. Irradiation of tephritid pupae under low-oxygen atmosphere (i.e., hypoxia) has been deemed mandatory to achieve sterility while preserving the quality and competitiveness of the sterile males. The objective of this study was to establish the radiation dose required to induce 99% percent sterility in *Z. cucurbitae* males irradiated as pupae under normoxic (air) and hypoxic atmospheres using gamma and X-ray sources.

Methods: Flies originated from a *Z. cucurbitae* colony established from wild material collected from infested fruits in Mauritius. Two sources of irradiation (Gamma and X-ray) were used. Hypoxia was created by sealing the pupae in plastic bags and allowing the flies to reduce oxygen levels for one hour. Pupae were irradiated 48 hours before emergence with the following doses: 30, 50, 70, 80 and 100 Gy under normoxia and hypoxia. After irradiation, the pupae were placed in cages until adult emergence. Upon emergence males were caged with virgin fertile (non-irradiated) females. Mating between non-irradiated males and females served as controls. Each experimental unit consisted of a cage holding 15 pairs. Following mating, females were provided an oviposition device, and three samples of eggs were collected per cage. Nine replicates were performed for each dose and atmosphere. Dose-response curves based on log-logistic functions were fitted to sterility data to estimate the effective doses inducing 99% sterility (ED₉₉), with confidence intervals calculated using the sandwich variance estimator. The ED₉₉ values were then compared between the radiation sources and atmospheric conditions using effective dose ratio tests.

Results: Log-logistic dose-response models indicated that the ED₉₉ did not differ significantly between gamma and x-ray under normoxic or hypoxic conditions.

Conclusions: Our results agree with previous studies conducted under normal atmosphere. Further research evaluating the quality of the flies, such as capacity to survive under stress, flight ability and male sexual competitiveness are required to estimate optimal dose and conditions for irradiation.

Key words: Melon fly, Sterile insect technique, SIT

Fruit Fly Surveillance and Monitoring for Species Diversity and Distribution with Emphasis on *Bactrocera dorsalis* Towards Readiness for Sterile Insect Technique (SIT).

Ndoda B. Msibi, Siphephile Msibi

Department of Biological Science, University of Eswatini, Malkerns Research Station

Background:

Fruit flies (Diptera: Tephritidae) remain among the most economically significant pests affecting horticultural production and international trade. In sub-Saharan Africa, the invasive species *Bactrocera dorsalis* has become a major threat due to its high reproductive capacity, polyphagy and competitive displacement of indigenous species. Eswatini faces continued risk of introduction and establishment of this pest through trade and movement of host materials. Effective surveillance systems are therefore essential for early detection, determination of species diversity and distribution, and for supporting advanced pest management approaches such as the Sterile Insect Technique (SIT). Establishing baseline data is a critical step towards the development of area-wide integrated pest management programmes and SIT readiness.

Methods:

A national fruit fly surveillance and monitoring programme was implemented across major agro-ecological zones, including agricultural production areas, markets and border posts. Trapping systems baited with male-specific attractants such as methyl eugenol, cue lure and trimedlure were deployed to target different fruit fly genera, with emphasis on *B. dorsalis*. Traps are serviced at regular intervals and specimens collected are preserved and identified using standard taxonomic procedures. Data will be analysed to determine species diversity, relative abundance and spatial distribution across the sampling sites.

Results:

Preliminary findings indicate the presence of diverse indigenous fruit fly species dominated by *Ceratitis* and *Dacus* species across production systems, with variations linked to host availability and agro-ecological conditions. There is widespread establishment of *Bactrocera dorsalis* in the lowveld part of the country; however, sporadic detections in other agro ecological zones have been detected. Species distribution patterns demonstrate areas of varying pest prevalence, providing critical baseline data for delimitation of areas of low pest prevalence and potential pest-free areas. The data further support the identification of high-risk zones requiring intensified monitoring.

Conclusions:

The surveillance programme has generated essential baseline information on fruit fly species diversity and distribution in Eswatini, forming a foundation for evidence-based decision-making in pest management. Continued monitoring is necessary to ensure mitigation strategies of *B. dorsalis* and to limit its establishment and spread. The results contribute directly to national and regional efforts towards the implementation of area-wide integrated pest management strategies and enhance the country's readiness for the application of the Sterile Insect Technique. Strengthening surveillance systems will be critical for safeguarding horticultural production, maintaining market access and supporting sustainable pest control programmes.

Key words: Fruit flies, *Bactrocera dorsalis*, surveillance, species diversity, distribution, Sterile Insect Technique, SIT readiness, Eswatini

Establishment and Functioning of a Sterile Medfly Unit in Morocco

Siham BOUJDIG, Anass EL AMRANI

National Office of Food Safety, Avenue Hadj Ahmed Cherkaoui, Agdal, Rabat, Morocco, Email: siham.boujdig@onssa.gov.ma

Background: The Mediterranean fruit fly, *Ceratitis capitata*, remains one of the most destructive pests affecting citrus production in Morocco, causing significant economic losses and posing a major constraint on exports. As part of efforts to strengthen Morocco's national integrated pest management strategy, the Sterile Insect Technique (SIT) was adopted because of its proven effectiveness and environmental sustainability.

Methods: Introduced in Morocco in 2006, this technique was first implemented through a programme involving the release of sterile male pupae in a 4,600-hectare pilot area in the Souss-Massa region. In 2017, the programme was extended to the Berkane region over 1,200 hectares, bringing the total treated area to 5,800 hectares. In light of the encouraging results obtained in reducing pest populations, the development of a national production capacity became a priority.

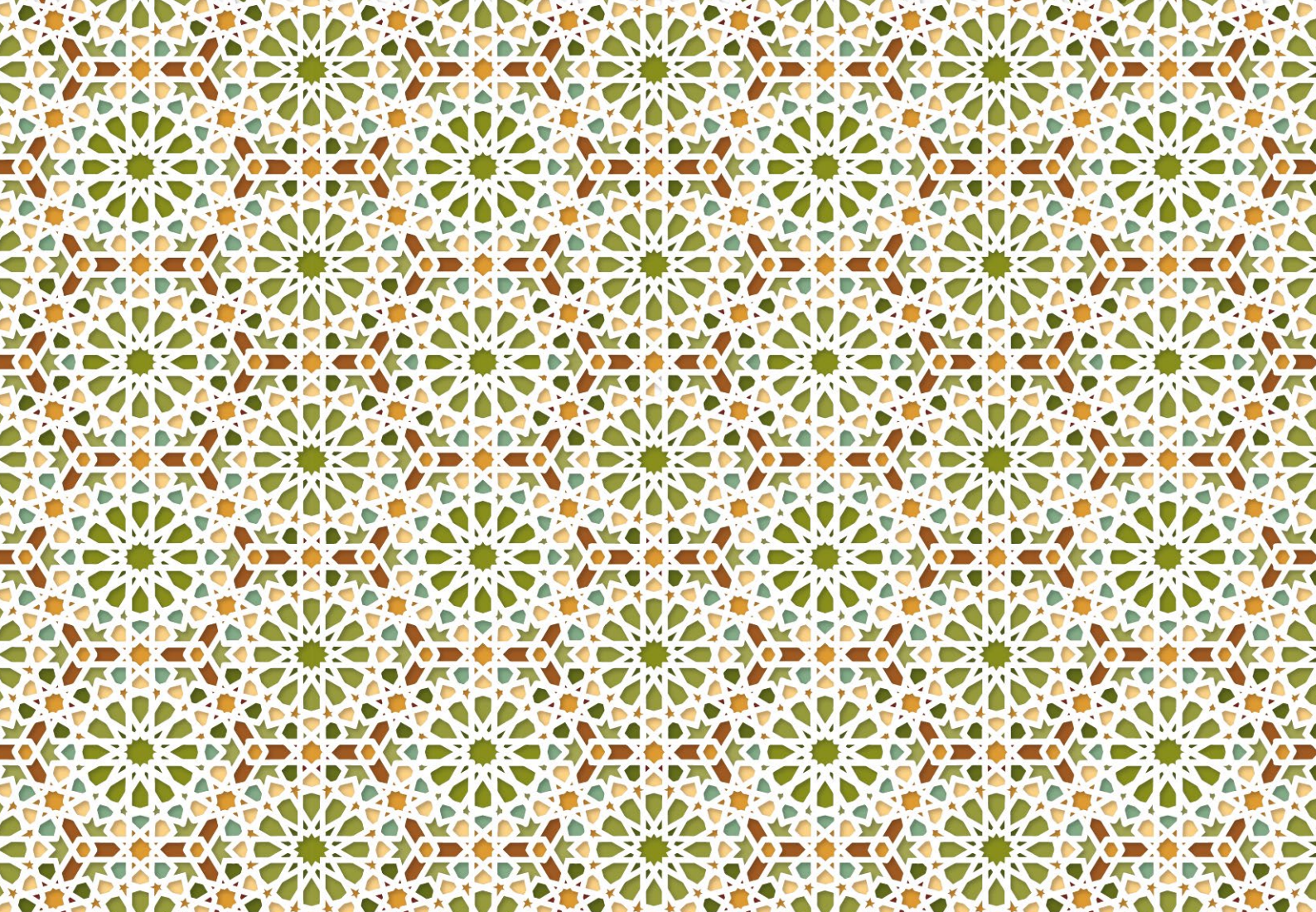
To this end, a facility dedicated to the mass rearing of sterile male Mediterranean fruit flies was established through the construction of the required infrastructure, the acquisition of rearing equipment, and the importation of a cobalt-60 irradiator for pupal irradiation. In parallel, specialised training was provided to strengthen the technical capacities of staff involved at the different stages of production.

Results: The unit is currently operational under the management of Morocco Citrus, the Moroccan interprofessional citrus organisation, with an estimated production capacity of 15 to 20 million sterile pupae per week. The support of public institutions, notably the National Office of Food Safety (ONSSA), the technical assistance of the International Atomic Energy Agency (IAEA), and the active involvement of the private sector were key factors in the successful implementation of this project.

Conclusions: In the long term, the objective is to increase production capacity to more than 350 million sterile pupae per week, in order to extend sterile male releases to all citrus-growing regions of Morocco within the framework of a sustainable and integrated pest management strategy. The establishment of this unit therefore represents a major step towards national self-reliance in the production of sterile insects and an important lever for strengthening the phytosanitary protection of Moroccan citrus in an environmentally sustainable manner.

Keywords: Mediterranean fruit fly, *Ceratitis capitata*, Sterile Insect Technique, mass rearing, irradiator, Morocco, integrated pest management.

Session 6. Sterile Insect Technique



POSTERS

Natural Enemies and Biological Control

Potential of soil-applied *Beauveria hoplocheli* for the biological control of *Bactrocera dorsalis* (Diptera: Tephritidae)

Abir Hafsi, Adriana Mussard, Irénée Promi, Magali Hoarau & **Laurent Costet**

CIRAD, UMR PVBMT, St Pierre, La Réunion F-97410, France.

E-mail : laurent.costet@cirad.fr

Background: *Bactrocera dorsalis* is one of the most widespread and damaging species, infesting a wide range of commercially important fruit and vegetable crops in tropical and subtropical regions. Control of this pest is challenging because its developmental stages occupy different microhabitats, particularly the soil-dwelling pupal stage. Entomopathogenic fungi (EPFs), particularly species of the genera *Beauveria* and *Metarhizium*, represent a promising alternative, as they infect insects by contact and can reduce adult emergence.

Methods: This study aimed to evaluate under field conditions the effect of different doses of the mycoinsecticide Betel, based on spores of *Beauveria hoplocheli*, as soil treatments on *B. dorsalis*. Field experiments were conducted in mango orchards across five parcels in La Réunion using three treatments: soil treated with 10× the recommended dose, soil treated with 100× the recommended dose, and an untreated control, each replicated three times. Twenty laboratory-reared L3 larvae were released into prepared soil plots and covered with emergence traps. Adult emergence was monitored daily. Emerged adults were transferred to the laboratory to assess survival and fungal infection over 30 days.

Results: The effects of *B. haplocheli* on adult emergence, and adult survival and *B. dorsalis* mycosis are discussed.

Conclusions: The results are expected to contribute to the development of sustainable control strategies targeting soil-dwelling stages of fruit flies.

Key words: Tephritidae, biological control, *Beauveria*, mycoinsecticide, survival, mycosis

Field release of *Diachasmimorpha longicaudata* using pupa-based devices as an alternative to conventional release systems

Amanda Ayala¹, Guadalupe García¹, David Sánchez¹ & Jorge Cancino²

¹Programa Moscas de la Fruta SENASICA-SADER, Camino a los Cacaotales S/N, PC 30860, Metapa de Domínguez, Chiapas, Mexico. ²Facultad de Ciencias Agrícolas, Universidad Autónoma de Chiapas Huehuetan Chiapas. Mexico. E-mail: amanda.ayala.i@senasica.gob.mx

Background: Augmentative releases of parasitoids are a fundamental component of area-wide integrated pest management (AW-IPM) programs against fruit flies. *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) is widely used in Mexico and is traditionally released using the “Arturito” device, which allows adult emergence under controlled conditions. However, this system presents operational limitations related to cost, transport volume, infrastructure requirements and reduced flexibility for releases in remote or resource-limited areas. Pupa-based release strategies, in which parasitoids emerge directly in the field from protected containers, may offer a practical alternative.

Methods: Fourteen-day-old pupae of *D. longicaudata*, close to adult emergence, were obtained from the Moscafrut mass-rearing facility and loaded into two alternative devices: modified PET bottles and cylindrical plastic containers (“vitroleros”), using different pupal densities. Adult emergence and internal mortality were evaluated under semi-field conditions. The most efficient device–density combination was selected for open-field evaluation and compared with the conventional “Arturito” system. Parasitism was assessed using sentinel traps containing irradiated *Anastrepha ludens* larvae, and parasitism levels were statistically compared between release methods.

Results: Adult emergence differed significantly among device–density combinations. The vitrolero device loaded with 5,000 pupae showed high emergence rates and the lowest internal mortality, indicating suitable ventilation and protection under field conditions. In open-field evaluations, parasitism levels obtained with the vitrolero-based release were not significantly different from those achieved using the “Arturito” device. High ambient temperatures recorded during the study period may have influenced overall parasitism levels; however, no differential effect between release systems was detected.

Conclusions: Pupa-based release devices represent a technically viable and operationally efficient alternative for the field release of *D. longicaudata* in AW-IPM programs. Their use can reduce costs, simplify transport and deployment, and increase operational flexibility without compromising field performance. These devices are particularly suitable for large-scale releases in areas with limited infrastructure or difficult access.

Key words: *Diachasmimorpha longicaudata*, augmentative biological control, pupa-based release, operational efficiency, fruit flies

~~Session 7. Natural Enemies and Biological Control~~

Screening of highly effective Entomopathogenic fungi and improvement of their control efficiency against *Bactrocera dorsalis* in Hainan Province

Baihui Hao^{1, 2} & Zhihong Li^{1, 2, *}

1 State Key Laboratory of Agricultural and Forestry Biosecurity, MARA Key Laboratory of Surveillance and Management for Plant Quarantine Pests, College of Plant Protection, China Agricultural University, Beijing 100193, China. 2 Sanya Institute of China Agricultural University, Sanya 572025, China. E-mail: lizh@cau.edu.cn

Background: The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a destructive invasive pest globally. Currently, its management relies largely on synthetic insecticides, which has led to severe resistance and environmental concerns. In Hainan Province, green control efforts face two major obstacles: the difficulty in controlling subterranean life stages (pupae and mature larvae) and the rapid inactivation of microbial agents due to intense solar ultraviolet (UV) radiation. While entomopathogenic fungi (EPF) offer ecologically compatible alternatives, their field efficacy is critically constrained by these environmental and physiological factors.

Methods: This study aims to develop a "dual-optimization" strategy to establish a robust all-life-stage control system against *B. dorsalis* in Hainan Province, focusing on enhancing pathogenicity against resistant subterranean stages and improving UV persistence for surface applications targeting adults. We screened twelve fungal isolates for virulence, evaluated binary strain combinations against larvae and pupae using a chi-square interaction model, and assessed the compatibility and protective efficacy of natural UV additives on conidial germination and adult mortality.

Results: Three highly virulent strains (*Metarhizium guizhouense* WN_M1/WN_M2 and *Beauveria bassiana* DF_B1) were selected. Binary combinations of these strains demonstrated stable additive effects, significantly increasing mortality rates in subterranean stages compared to single isolates. Furthermore, the incorporation of humic acid (Sodium/Potassium Humate) maintained conidial germination rates >85% after severe UV-B exposure (24 h post-irradiation), while unprotected controls remained below 35%. This formulation also significantly preserved virulence against adults under simulated solar stress.

Conclusions: This study employs a dual-optimization strategy combining optimized fungal strain consortia with UV-protective formulations to effectively overcome key limiting factors facing fungal biocontrol across different developmental stages of *B. dorsalis*. These findings provide an effective new strategy for constructing a sustainable Integrated Pest Management (IPM) system for this pest.

Key words: *Bactrocera dorsalis*, entomopathogenic fungi, UV protection, sustainable pest management

EFFECTS OF THE ARTIFICIAL LARVAL DIET AND HOST SPECIES ON QUALITY PARAMETERS OF *DIACHASMIMORPHA LONGICAUDATA* (HYMENOPTERA: BRACONIDAE)

Francisco Devescovi¹, Xiomara Montealegre¹, Adriana Pazos², Gabriela Gallardo², Sebastián Cunzolo², Micaela S. Garbalena¹, Franco Pacelli¹, Ana Nussenbaum^{1,3} & Diego F. Segura^{1,3}.

1 Instituto de Genética “E.A. Favret”, INTA, GV-IABIMO, CONICET. Hurlingham, Buenos Aires, Argentina.

2 Instituto de Ciencia y Tecnología de Sistemas Alimentarios Sustentables, UEDD INTA CONICET, Hurlingham, Buenos Aires, Argentina.

3 Instituto de Investigación en Ciencias Agrarias y Veterinarias, USAL, Argentina
e-mail: devescovi.francisco@inta.gob.ar

Background: In Argentina, *Ceratitis capitata* (Cc) and *Anastrepha fraterculus* (Af) are two pest species that cause great losses for the fruticulture. *Diachasmimorpha longicaudata* (Dl) has been proposed as an adequate biological control agent for IPM programs. This is a solitary, sinovigenic, koinobiont endoparasitoid. In the laboratory, we artificially rear these three species with experimental purposes. For each fly species, we use specific artificial larval diets (Diet_{Cc} and Diet_{Af}) that maximize the rearing yield of each colony. For the rearing of Dl, adults parasitize L3 larvae that are exposed in artificial oviposition units mimicking infested fruit. Our aim was to determine a combination of host and larval diet that maximizes the biological quality of adult parasitoids.

Methods: Using a factorial design with the two fruit fly species and their respective larval diets we obtained four types of parasitoids (reared on: Cc-Diet_{Cc}, Cc-Diet_{Af}, Af-Diet_{Af}, Af-Diet_{Cc}) that were evaluated in their developmental time (egg-adult), flight ability, survival (starvation/fed), fecundity, and parasitism %, among others. For each dependent variable, the results were used to rank these four treatments. As explanatory variables, we measured wing size (length and width), the weight of host pupae, and determined the nutritional composition of the different types of diets, larvae, and resulting parasitoids.

Results: The overall quality ranking obtained with the use of different combinations of host species and larval diet indicated Af-Diet_{Af} as the most promising. This was mainly due to the best results in flight ability, female proportion in the offspring, survival (starvation), and wing size, and secondarily to fecundity, percentage of parasitism, and survival (fed). Intermediated scores were achieved by Af-Diet_{Af} and Cc-Diet_{Af}. The lowest scores were obtained when Cc-Diet_{Cc} was used for parasitoid rearing.

Conclusions: this ranking may be explained by the higher energy content measured in adult wasps after developing in *A. fraterculus* hosts (independently of the larval diet) and by the bigger size (nutritional amount) of this host. The patterns of macronutrients and energy in the diets and larval types still do not allow us to conclude how this higher energy content in adult wasps is obtained. Further aspects about the physiological mechanisms need to be considered to answer this question.

Key words: Biological control, fruit flies, artificial rearing, optimization, quality parameters.

Influence of altitude on the infectivity of *Beauveria bassiana* against *Ceratitis capitata* in Chiapas, Mexico

Guadalupe García-Coapio¹, David Sánchez Robledo¹

¹ Moscamed Programme - Mexico, SADER-SENASICA, Camino a los Cacaotales S/N, Metapa de Domínguez, Chiapas, C.P. 30860, México.

E-mail: coapioguadalupe@gmail.com

Background: *Ceratitis capitata* remains a major pest across tropical and temperate zones. In Moscamed's region (0–1600 m above sea level), significant variations in temperature and humidity can affect the performance of entomopathogenic fungi. We investigated how altitude and seasonal conditions affect the infectivity of *Beauveria bassiana*, a crucial factor for broad-area pest management. Understanding how microclimatic factors at different altitudes influence fungal pathogenicity is vital for optimizing auto-inoculation strategies. This is especially important in tropical mountain environments, where daily thermal fluctuations, solar radiation, and seasonal shifts in humidity can accelerate conidial degradation or alter host susceptibility. By evaluating *B. bassiana*'s effectiveness at different altitudes, this research aims to establish operational guidelines to enhance the biological control of *C. capitata* under real field conditions.

Methods: Sterile male flies (Vienna 8 TSL) were exposed to *B. bassiana* (approx. 2×10^9 conidia g⁻¹) or kept as non-inoculated controls, then released at three sites: Metapa (99 m), Cacahoatán (547 m), and Unión Juárez (around 1365 m). Survival was tracked during dry and rainy seasons using Kaplan–Meier and Log-rank tests (estimating LT₅₀). Simultaneously, auto-inoculation devices (yellow flannel panels) were tested under shade and sun after 0, 8, 15, and 30 days outdoors, measuring conidial viability at each interval.

Results: In the dry season, inoculated flies had a lifespan roughly 50% shorter than controls (LT₅₀ = 7–13 days vs. 22 days), with quicker and steeper survival declines at mid-altitude. During the rainy season, overall survival decreased (controls around 10 days; inoculated LT₅₀ = 5–7 days). The infectivity of devices declined sharply with field exposure and was nearly lost by day 30, paralleling the drop in conidial viability. Freshly impregnated panels consistently showed the lowest LT₅₀, with shade conditions reducing performance loss compared to sun exposure.

Conclusions: Environmental factors such as temperature, humidity, and solar radiation significantly influence the effectiveness of *B. bassiana* across different altitudes. In practice, re-impregnating devices within 8 days and installing them in the shade is recommended. Developing more virulent strains and improved formulations may help maintain efficacy under diverse field conditions.

Keywords: entomopathogenic fungi, altitude, auto-inoculation device, infectivity, survival.

**Environmental and Temporal Effects on the Release of Parasitized Pupae of
Doryctobracon areolatus (Hymenoptera: Braconidae)**

Kevin Suarez-Buitrago¹, Roberta Manica Berto², Sandro Nörnberg², Rafael Gonçalves², Natalia Ladino³, **Lorena Quintero-Rodríguez¹**, Thiago Mastrangelo⁴ & Dori E. Nava¹

¹EMRAPA Clima Temperado, BR 391 km 78, Pelotas, 96010-971, RS, Brazil.

²PARTAMON Parque Tecnológico Room 4i, Av. Domingo de Almeida 1785, Pelotas, 96085-470, RS, Brazil.

³Federal University of Pelotas (UFPel), Capão do Leão, 96010-610, RS, Brazil.

⁴Centre for Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP), Av. Centenario 303, Piracicaba, 13416-000, SP, Brazil. E-mail: lquinterorodriguez163@gmail.com

Background: *Anastrepha fraterculus* (Diptera: Tephritidae) is native to the New World, with approximately 167 host plants recorded in Brazil. Although its control relies mainly on chemical insecticides, the larva–pupal parasitoid *Doryctobracon areolatus* (Hymenoptera: Braconidae) frequently parasitizes *A. fraterculus* larvae, reaching parasitism rates of up to 53%, highlighting its potential as a biological control agent. However, mass production and release of adults present operational limitations for large-scale application. As an alternative, the release of parasitized pupae has been proposed, but biotic and abiotic challenges associated with this strategy must be evaluated.

Methods: Transects were established in an experimental *Prunus persica* orchard and in its adjacent conservation area at EMBRAPA Clima Temperado to compare predation rates, parasitoid emergence after 3 hours of exposure, and the composition of soil-dwelling predators during the morning (8:30–11:30) and afternoon (12:00–15:00) periods. In addition, the use of biodegradable capsules was evaluated as a strategy to increase the survival of parasitized pupae.

Results: The highest mean predation rates were recorded during the afternoon period (54%) and in the adjacent conservation area (71.8%). Nevertheless, adult emergence was higher in this area, with a mean of 34 individuals, compared with the center of the orchard, where a mean of 10 individuals was recorded. Regarding the evaluation period, emergence was higher in the morning (27 individuals) than in the afternoon (18 individuals). Soil-dwelling predators in both environments were mainly represented by ants of the genera *Pheidole* and *Solenopsis*. The use of biodegradable capsules increased repellency in both environments compared with the control (pupae without capsules), particularly in the adjacent conservation area and during the afternoon period.

Conclusions: The results indicate that the release of parasitized pupae is more efficient during the morning period and in shaded environments, close to the time of adult parasitoid emergence. Ants were identified as the main predators. The use of biodegradable capsules increased pupal viability against biotic factors.

Key words: South American fruit fly, biological control, predation, parasitized pupae.

Habitat-scale chemical landscapes shape *Fopius arisanus* parasitic activity

Vincent Jacob, David Arzac, Laura Moquet & H       Delatte

CIRAD, UMR PVBMT, 7, ch. de l'IRAT, F-97410 Saint-Pierre, La R        , France

E-mail: vincent.jacob@cirad.fr

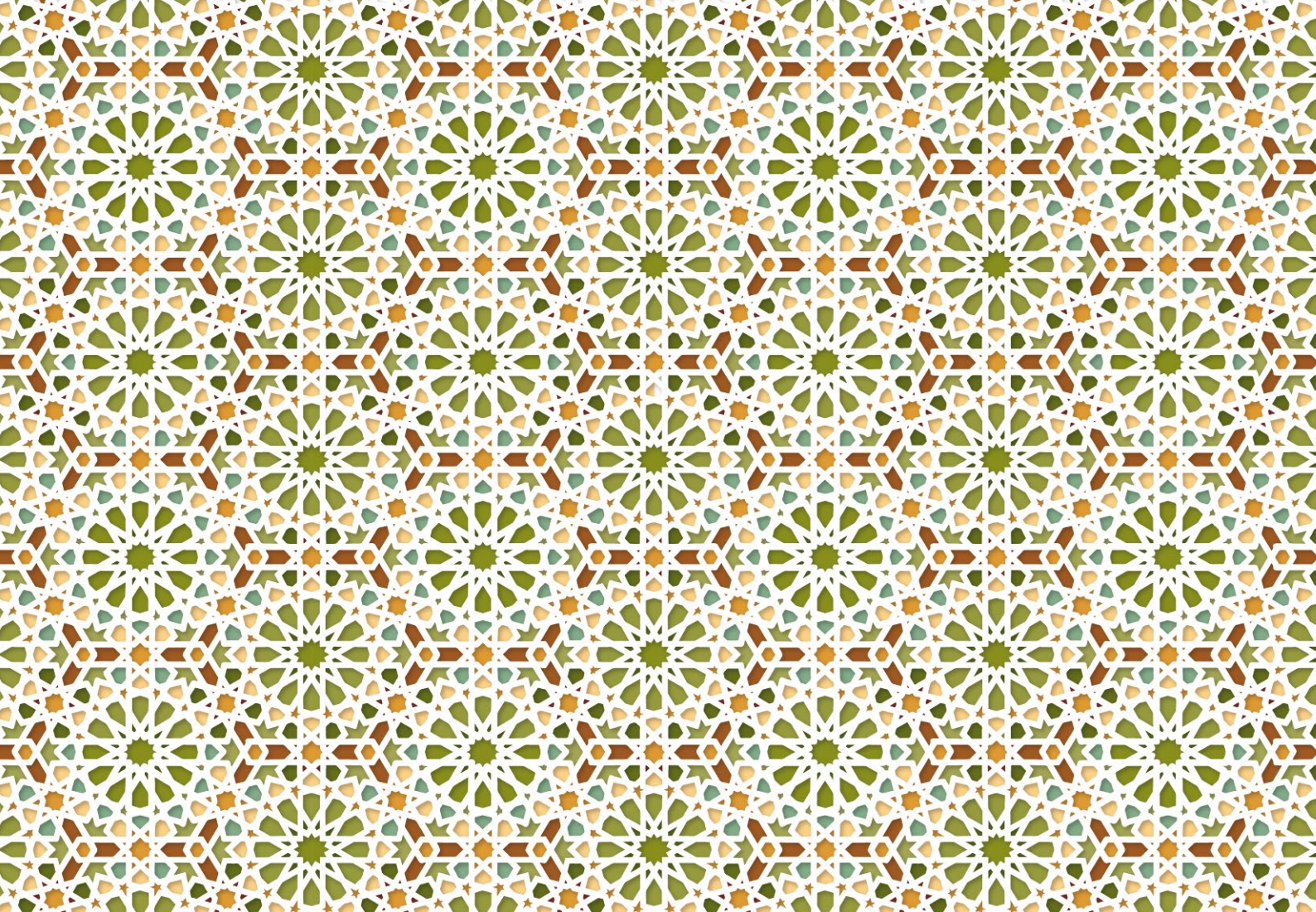
Background: Parasitoid foraging is classically viewed as being driven by host- and associated plant-derived semiochemicals. However, chemical cues operating at the habitat scale may modulate these responses by shaping the olfactory background against which hosts are detected, potentially reducing their effectiveness as biological control agents. Here, we investigated such background semiochemicals shaping *Fopius arisanus* parasitic activity using field experiments and *ex situ* assays.

Methods: The spatiotemporal variability of *F. arisanus* parasitism activity and environmental volatile organic compound (VOC) profiles was assessed simultaneously across four mango orchards over four seasons, with three consecutive weeks of sampling per season and multiple replicates of sentinel *Bactrocera dorsalis* eggs and Tenax air samples per orchard and per week. VOCs whose presence covaried with high parasitism were subsequently tested in laboratory oviposition assays.

Results: *F. arisanus* parasitism occurred year-round at low levels and peaked during mango fruit maturation. During this season, parasitism intensity varied markedly across orchards, ranging from 0% to 40% of parasitized sentinel eggs. High parasitism was associated with distinct VOC profiles that deviated from the generic mango orchard volatile signature. In *ex situ* assays, one compound consistently stimulated *F. arisanus* oviposition upon contact, whereas another significantly inhibited oviposition even at a distance.

Conclusions: Semiochemicals reflecting plant and habitat conditions covaried with *F. arisanus* activity in the field and may contribute to the parasitoid attraction or deterrence. Two compounds were functionally validated as direct modulators of *F. arisanus* oviposition behaviour in opposite directions. Together, these results demonstrate that parasitoid foraging decisions are embedded in the surrounding habitat-scale chemical landscape. This insight opens new avenues for improving biological control by leveraging identified semiochemicals to enhance parasitoid efficiency.

Key words: Biological control, Semiochemicals, field study, Oviposition behaviour



POSTERS

Other Control Methods

Development and Field Application of the TELEMO Fruit Fly Intelligent Monitoring System

Jinping DU, Xinghui LIANG, Qing DENG, Xueliang SUI, Xuefei JIN

Beijing Ecoman Biotech Co., Ltd, Unit 518, No.26, Information Road, Shangdi, Haidian district, Beijing, China. Email:dujp@ecomanbiotech.com

Background: Fruit flies, particularly *Bactrocera dorsalis* (Diptera: Tephritidae), inflict substantial economic losses on horticultural crops through direct damage, reduced market value, and quarantine restrictions. Conventional monitoring relies on manual inspection, which is labor-intensive and limits timely control. Thus, a more efficient monitoring system is essential for effective monitoring of fruit flies.

Methods: The TELEMO intelligent fruit fly monitoring system was developed to enable real-time detection and monitoring of fruit fly populations. The system integrates high-performance infrared sensors for automated insect detection and counting. Field trials were conducted in mango orchards located in Tandou Town, Leizhou City, Guangdong Province, China. At each test site, three TELEMO prototypes were deployed, with each unit responsible for monitoring an area of 7 hectares. The target species was the oriental fruit fly, *B. dorsalis*. Both TELEMO devices and control traps were used with methyl eugenol (ME) as lure. Traditional traps were served as control. Daily manual count was conducted for 14 consecutive days. During each inspection, trapped insects in both TELEMO devices and traditional traps were manually counted and identified. Counting accuracy was determined by comparing the system's automated counts with manual counts from the TELEMO traps. Trapping efficiency of TELEMO was assessed relative to traditional traps. Data timeliness was measured as the interval between insect detection and successful synchronization to the remote server. Economic benefits were evaluated by comparing labor requirements between the two monitoring methods.

Results: Field trials demonstrated that the TELEMO system provided real-time insights into *Bactrocera dorsalis* population dynamics, with a maximum data synchronization delay of ≤ 5 seconds between detection and upload to the cloud server. The mean counting accuracy, based on comparisons between automated and manual counts from TELEMO traps, was 90.67% . Relative to traditional traps, TELEMO achieved a mean trapping efficiency of 95.02% . Overall labor costs for monitoring with TELEMO were 68.5% lower than those associated with traditional manual traps ($P < 0.05$).

Conclusions: These results indicate that TELEMO offers excellent data timeliness, high counting accuracy, and superior trapping efficiency compared to conventional methods, while substantially reducing labor requirements. The TELEMO system advances this field by employing infrared sensors for unsupervised, real-time field monitoring, significantly reduces operational costs and provides timely, reliable data to support evidence-based fruit fly prevention and control strategies.

Key words: TELEMO Fruit Fly Intelligent Monitoring, Counting Accuracy, Trapping Efficiency, Cost Reduction

Evaluation of Lure-&-Kill panels for *Ceratitis capitata* control under different environmental conditions

Valeria Papili¹, Eleftheria Maria Bali², Marco Colacci¹, Parhs Prekas², Nikolaos Papadopoulos², Andrea Sciarretta¹

¹Department of Agricultural, Environmental and Food Sciences, University of Molise, Via de Sanctis, Campobasso, Italy

²Laboratory of Entomology & Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, School of Agricultural Sciences, University of Thessaly, Volos, Greece

E-mail: v.papili@studenti.unimol.it

Background: The Mediterranean fruit fly, *Ceratitis capitata*, is one of the most destructive pests of fruit crops worldwide. Control has traditionally relied on synthetic insecticides such as spinosyns, neonicotinoids, and pyrethroids. Within an Integrated Pest Management (IPM) framework, more sustainable approaches such as Lure-&-Kill (L&K) have been developed. This technique uses attractants combined with insecticides to lure and kill adult flies. However, the performance of L&K devices under varying environmental conditions remains poorly understood. The aim of the current paper was to assess the effect of field aging and different environmental conditions on the efficacy of BioMagnet Amber (Suterra) against adults of the Mediterranean fruit fly.

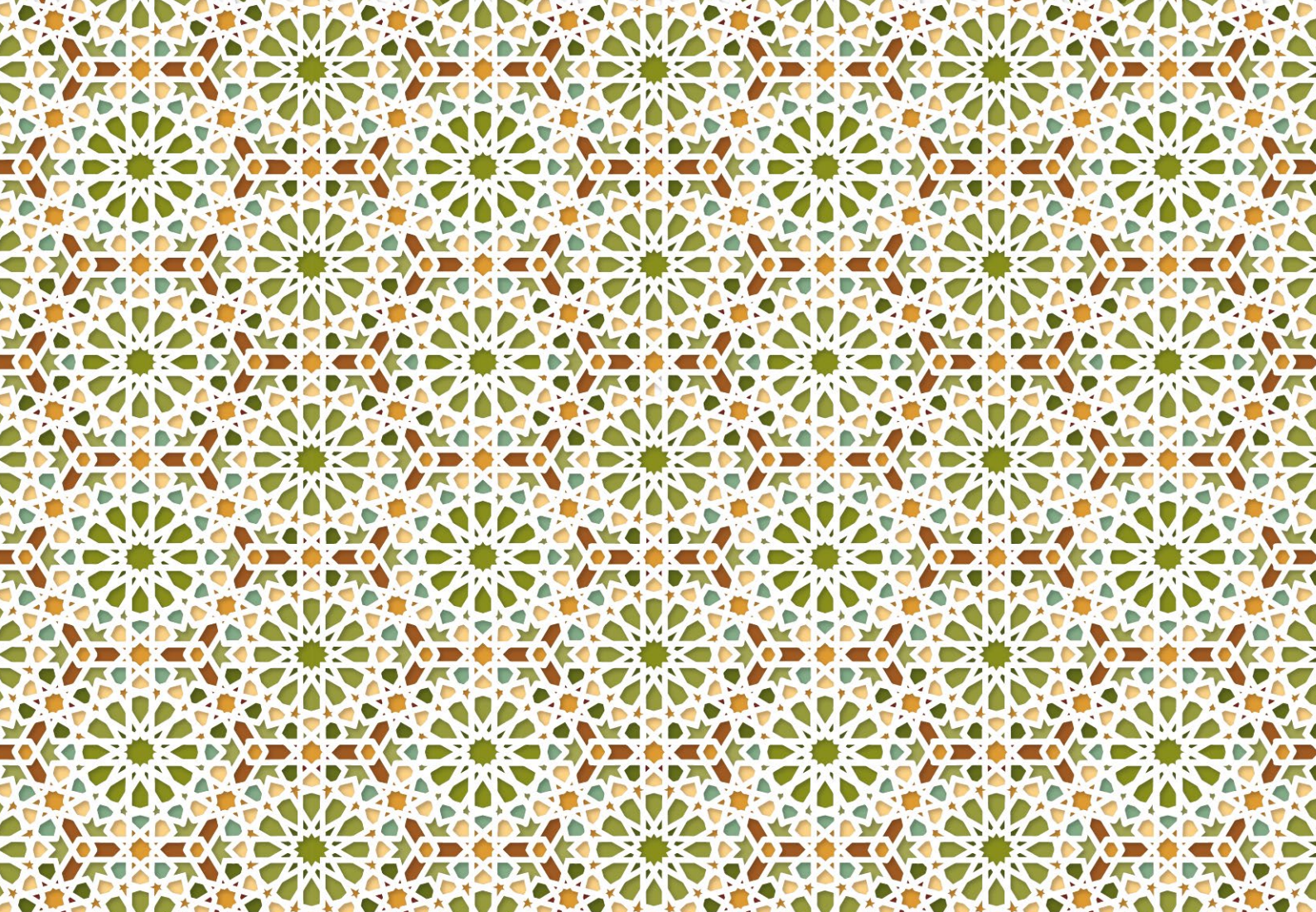
Methods: Trials were performed in six cylindrical plastic-screen field cages (2.9 m diameter × 2 m height), each housing one potted citrus tree. In each cage one panel was placed. Panels were exposed to five different conditions: (1) sunlight exposure, (2) simulated rainfall, (3) shade, (4) natural conditions (inside tree canopy in field conditions), (5) fresh panel (as positive control). As negative control, a white paper sheet with no attractant and killing agent was used.

In each cage, 100 mixed-sex (50 males and 50 females), 7-10 days old adults were released. Devices were exposed to the various conditions at the end of May, in order to test effect of panel aging. Five replications were performed in July with 2 months old panels, and in September with 4 months old panels. The surviving flies in the cage were recorded after 24 and 48 hours exposure to BioMagnet panels.

Results: The efficacy of the L&K panels over 4 months was affected by the environmental conditions. At 2 months of aging, all treatments reduced male and female survival compared to the negative control, particularly within the first 24 hours, with no substantial differences among the tested treatments. After 48 hours, survival was generally low across all treatments, confirming sustained device efficacy over time. At 4 months of aging, significant differences among treatments were found. Survival rates were higher in the negative control and the sun-exposed devices compared with all other treatments. Conversely, survival was significantly lower in the positive control, rain, shadow, and natural condition treatments at both 24 and 48 hours.

Conclusions: The results demonstrated that the prolonged sun exposure reduce the efficacy of L&K panels, whereas other environmental conditions has rather moderate negative effect on the performance of L&K devices.

Key words: *Ceratitis capitata*, Mediterrean Fruit Fly, Lure-&-Kill



POSTERS

Area-wide Integrated Pest Management and Action Programs

Impact of the *Bactrocera oleae* infestation on the total polyphenol content of two varieties of olives: the Picholine Marocaine and the Picholine Languedoc

Aziz Ait Si Mbarek^a, Abderrahim El Keroumi^a, Ismaïl Outoukarte^b, My Ahmed Al Aloui El Fels^a.

^aLaboratoire de pharmacologie, neurobiologie, anthropobiologie et environnement, Faculté des sciences Semlalia, Université Cadi Ayad, Bd. Prince My Abdellah, B.P. 2390, 40000 Marrakech, Maroc, E-mail: a.aitsimbarek.ced@uca.ac.ma

^bLaboratoire polydisciplinaire de recherche en didactique, Education et Formation, CRMEF Marrakech Safi.

Background: *Bactrocera oleae* (is the most destructive worldwide pest of olive fruits, that inflicts substantial quantitative and qualitative yield losses. To mitigate the impact of this economically significant pest, it is indispensable to implement an effective pest management strategies that consider a better understanding of biochemical responses in attacked and non-attacked olive fruits in cultivated olive varieties, and within bioclimatic areas of olive commercial production.

Methods: This study evaluated the effect of olive fly infestation on total polyphenol content in two predominant olive varieties (Picholine Marocaine and Picholine Languedoc) at two ripening stages (green and black) in two major production sites of the Marrakech/Tensift region (Attaouia and Amzmiz). Total phenolic content was quantified in infested and non-infested fruits using methanolic extracts and the Folin–Ciocalteu colorimetric method. Data were analyzed by multifactor ANOVA using SPSS software

Results: Total phenolic content ranged from 13.3 to 25.9 mg GAE/g FW. Infested fruits showed a strong decrease ($p < 0.001$), with losses of about 3–4 mg GAE/g FW in Picholine Marocaine and up to 4–8 mg GAE/g FW in Picholine Languedoc, depending on site and ripening stage. A significant genotypic effect ($p < 0.001$) was detected: non-infested Picholine Languedoc generally contained 19–24 mg GAE/g FW, while Picholine Marocaine mostly ranged between 15 and 18 mg GAE/g FW. Phenolic content also differed between ripening stages ($p < 0.001$), green fruits often having slightly higher levels than black fruits within the same variety and health status. No significant differences in mean phenolic content were found between sites, but the stronger depletion observed in Picholine Languedoc under infestation indicates that Picholine Marocaine is relatively more resistant in maintaining phenolic content under *B. oleae* attack.

Conclusions: The results of this study are essential for effective crop management and integrated pest management (IPM) strategies. By characterizing the total polyphenols in attacked and unattached fruit in olive varieties, our results provide empirical markers that could guide cultivar choice and help growers favor the relatively more resistant picholine Marocaine thereby reducing the risk of severe quality losses.

Keywords: *Bactrocera Oleae*, *Olea europaea*, Marrakech/Tensift region, Total polyphenol contents.

"Design and Development of a Ground Release Equipment for Sterile Mediterranean Fruit Flies (*Ceratitis capitata*) using the Chilled Adult Technique in Argentine Patagonia"

Mongabure, Alejandro¹, Musi, Alejandro¹, Stazionati, Claudia¹, Borges, Carlos Alfredo¹ Rodríguez Facundo¹, Di Meglio Mauro¹, Sarapura, Federico¹ & Piermattei, Lorena¹. Merayo Eduardo², De Azevedo Pablo³

²Fundación Barrera Zoofitosanitaria Patagónica (Funbapa); ²Director and ³Deputy Director (Funbapa)

amongabure@funbapa.org.ar; alejandromusi@funbapa.org.ar

Argentine Patagonia has maintained its status as a Fruit Fly Free Area since 2005, with national and international recognition, a key condition for the production and export of pome and stone fruits in its irrigated valleys. The Fruit Fly Program in Patagonia is executed by the Patagonian Zoophytosanitary Barrier Foundation (FUNBAPA) and implements systematic releases of sterile males of *Ceratitis capitata* in urban areas, using the Sterile Insect Technique (SIT) as a key tool for preventive control.

The design and development of prototypes of specialized machines for ground release in smaller-area localities began in 2020. The chilled sterile flies are loaded into a hopper on the device, which the machine disperses, controlling their release density. Initially, this system was basic and rudimentary, although satisfactory results were achieved in terms of recapture in traps, expressed as Sterile Flies/Trap/Day (FTD). In 2023, the National Institute of Industrial Technology of Argentina (INTI, by its acronym in Spanish) was asked to improve the method by automating it, which allowed for an increase in the release area. In 2024, a smartphone application was developed with the goal of quantifying the releases carried out.

Currently, each machine can release 1.5 million chilled adults in 3 hours and have a specific hardware and software system that incorporates geolocation, automatic dosage, operational logging, and insect control in the hopper, providing the operator with real-time information on the progress of the release. These improvements make it possible to optimize the efficiency, traceability, and precision of the Chilled Adult ground release process.

Keywords: *Ceratitis*; ground release devices; SIT; Patagonia

Technological Innovation in Emergence Rooms for Sterile *Ceratitis capitata* (Wiedemann) Males using the Chilled Adult Technique in Allen, Argentine Patagonia

Mongabure, Alejandro¹, Musi, Alejandro¹, Stazionati, Claudia¹, Borges, Carlos Alfredo¹, Rodríguez Facundo¹, Di Meglio Mauro¹, Sarapura Federico¹ & Lorena Piermattei¹, Merayo Eduardo², De Azevedo Pablo³

²Fundación Barrera Zoofitosanitaria Patagónica (Funbapa); ²Director and ³Deputy Director (Funbapa).

amongabure@funbapa.org.ar; alejandromusi@funbapa.org.ar

The Chilled Adult Emergence Center (CEDAF: *Centro de Emergencia de Adulto en Frío*, by its acronym in Spanish) is located in the town of Allen, Río Negro Province, Argentina. It implements a standardized process for the packaging, conditioning, quality control, emergence, and release of sterile *Ceratitis capitata* (Wiedemann) males using the Chilled Adult Technique (CAT), as part of the Fruit Fly Program in Argentine Patagonia.

The packaged emergence towers are transferred to rooms under controlled temperature and humidity conditions until the adults emerge.

As part of this process, two new emergence and thermal shock (knockout) rooms were designed, constituting a technological innovation in the application of the CAT. These rooms are characterized by their high response capacity, allowing for the simultaneous reduction of relative humidity and temperature in a short period of time within the same space. The integrated dehumidification system makes it possible to decrease relative humidity from values of 65% to 30% in the same enclosure, ensuring the reduction of condensation and, consequently, the clumping of insects during this process.

The automated thermal control allows for the programmed temperature to drop from 21°C to 0°C in approximately 40 minutes, ensuring the rapid and precise application of the thermal shock necessary for insect immobilization, thereby reducing the time to cold stress (physiological damage).

The system is complemented by remote monitoring and control of environmental parameters (temperature, humidity, and lighting), through a mobile app; and also includes alarms for critical deviations, and light timers that simulate diurnal and nocturnal cycles. The combination of these features optimizes the conditioning of adults, reduces operational times and handling, and contributes to improving the efficiency of the process and the biological quality of the sterile males released.

This technological innovation positions “CEDAF Allen” as a regional reference in the application of automated systems for the Chilled Adult Technique, strengthening process standardization, operational efficiency, and the quality of the sterile insects released.

Keywords: *Ceratitis*; emergence rooms; chilled adult technique; Patagonia

Area-Wide Management for Fruit Flies in Java Island – Moving or Stagnant?

Andi Abdurahim

Directorate General of Horticulture, Ministry of Agriculture, Jakarta, Indonesia.

Email: mr.andy.162@gmail.com

Background: Since the launch of the Indonesia-Australia Area-Wide Management (AWM) program for fruit flies (*Bactrocera dorsalis*) in 2010, AWM has become the generic name for fruit fly eradication programs in Indonesia. It applies to any type of fruit, any location, and any area. From 2010 to 2022, AWM on mango plants in West Java Province has yielded quite satisfactory results (fly numbers at less than 1 fly/trap/day). However, there are several points that need to be addressed. Frankly, AWM cannot yet offer "instant" results like the use of chemical insecticides. Achieving AWM requires a considerable amount of time and complete and accurate data collection. This complete and accurate data will be the starting point for determining whether AWM will be further developed. Farmer group institutions also do not yet have a strong bargaining position to assert that they – farmers – are the core implementers of AWM and all its instruments. Policy makers from the Central Government have not been able to map the land area, land position, and types of commodities that will be the focus of the implementation of AWM.

Methods: Policymakers from the central and regional governments exchanged ideas to bridge the needs of farmer groups, business actors, and the wider community regarding fruit quality. The institutional structure of farmer groups targeted by the AWM program was mapped based on their needs and capabilities. Technical guidance was held periodically (online and offline) at several AWM target locations.

Results: Policymakers from the central and regional governments have a clear understanding of how AWM can be implemented effectively. Farmer groups have been actively involved in implementing AWM for a relatively long period of time. Horticultural industries are beginning to consider the quality of the fruit resulting from AWM implementation.

Conclusion: Collaboration among stakeholder, horticultural industries and farmer group 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 Java.

Keywords: *Bactrocera dorsalis*, Fruit fly, Area Wide Management, AWM

MED FLY *CERATITIS CAPITATA* SUPPRESSION ON MAHE ISLAND IN SEYCHELLES

Samuel Brutus¹, Randy Stravens¹, Valerie Henri¹, Stephania Cedras¹, Denis Lesperance¹, Lilianne Cadeau¹, Mark Nicolas¹, Preaduth Sookar² & Rui Perreira³

¹Agriculture Department, Ministry of Agriculture, Climate Change and Environment, Victoria, Mahe, Seychelles

²Entomology Division, Ministry of Agro Industry and Food Security, Reduit, Mauritius

³Insect Pest Control, Joint FAO/IAEA Division on Nuclear Application for Food and Agriculture, Vienna, Austria

*Corresponding author: E-mail: brutussamuel@gmail.com

Background: The Mediterranean fruit fly, *Ceratitis capitata*, was introduced to Seychelles in 1900 and has become a major pest of Solanaceae, Citrus and other fruit crop across the main islands of the archipelago. After the introduction, the Seychelles government initiated a campaign to eradicate med fly in the country. However, due to technical and economic reasons, the objective was changed to suppression.

Method: The following suppression methods were integrated: (1) sanitation; (2) male annihilation technique (MAT) blocks with protein hydrolysate plus malathion; (3) bait spray with protein hydrolysate and (4) mass trapping with Trimedlure (TML). To assess the effectiveness of the suppression program, a network of 38 tephri traps using TML as an attractant was deployed and fruit sampling was carried out fortnightly. Adult populations captured in TML traps decreased from about 8 flies per trap per day (FTD) (to <1 FTD and fruit infestation reduced from about 95% at commencement to 35% within <month. Farmer's income increased due to more marketable fruits.

Conclusions:

The 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 is recommended to combine the above suppression methods with the Sterile Insect Technique (SIT), a strategy currently implemented in various parts of the world

Keywords: sanitation, male annihilation technique, bait sprays, bait stations, adult surveillance, larval surveillance.

Survey of fruit flies (Tephritidae: Dacini) in Qasr Bin Ghashir Municipality, Greater Tripoli Governorate, Libya

Esam Elghadi¹, Abdusalam Elgattaa¹, Hager Kehil² & Abdulmajeed Albaghdadi²

¹ Libyan Biotechnology Research Centre (LBTRC), National Project for Integrated Fruit Fly Management, Tripoli, Libya.

² The National Center for Prevention and Agricultural Quarantine, Libya

E-mail: esamelghadi@lbtrc.edu.ly, esam742002@yahoo.co.uk

Background: Tephritid fruit flies represent the greatest threat to fruit orchards and vegetable crops in Libya. The municipality of Qasr Bin Ghashir is one of the most important agricultural areas and is among the municipalities targeted by the fruit fly monitoring program implemented by the National Integrated Fruit Fly Management Project in cooperation with the International Atomic Energy Agency (IAEA). Monitoring and surveillance activities are the main measures in fruit fly management. The presence of fruit fly species was verified during a four-year monitoring program in the Qasr Bin Ghashir municipality from 2022 to 2025.

Methods: The study was conducted over four years, from January 2022 to December 2025, in fruit-growing areas of Qasr Bin Ghashir municipality in northwestern Libya. McPhail traps were used, separately baited with three male lures (trimed-lure, methyl eugenol and cue-lure). Also, fruit sampling was taken whenever possible from some of the trapping sites. The fruit flies obtained from the traps and fruits were identified based on the morphological characteristics of the specimens.

Results: Three fruit fly species of economic importance were reported including the Mediterranean fruit fly, *Ceratitis capitata*, the Greater melon fly, *Dacus frontalis*, and the Peach fly, *Bactrocera zonata*. The abundance of *Bactrocera zonata* at the survey sites confirms that this pest is of great economic importance in citrus production, and that it may be the species causing the highest percentage of citrus damage in the study area.

Conclusions: Overall, the exotic fruit fly *B. zonata* was most caught species in traps and in fruits followed by the native *C. capitata*.

Key words: Survey, fruit flies, traps, fruit sampling, Libya.

Computer Systems and Artificial Intelligence (AI) for Phytosanitary Surveillance in Argentina's National Fruit Fly Program (PROCEM)

Esteban Garavelli¹, Paola Y¹, M. Florencia Vazquez¹, Bernardo Repice¹ Héctor E. Medina¹

¹National Service for Agri-food Health and Quality (Senasa)– Av. Paseo Colón 367 - Autonomous City of Buenos Aires - Argentina. E-mail: procem@senasa.gob.ar

The National Service for Agri-food Health and Quality (Senasa), acting through the National Fruit Fly Program (PROCEM), has implemented a technological innovation that enhances the reliability of the official Phytosanitary Surveillance System. This technology consists of a real-time data recording system using mobile devices and QR codes associated with each trap, which are subsequently linked to an online platform known as SIGPROCEM. Through this system, the collection of data and associated variables—including trap identification, the officer responsible for the action, geographic coordinates, and the precise timing of field activities—is fully automated. As a complement to trapping, fruit sampling management is also integrated into the platform. This innovation eliminates the use of paper-based forms, thereby minimizing human error and significantly streamlining field operation lead times. Furthermore, it enables efficient consolidation of information, the determination of pest population levels within an Area-Wide framework, and the generation of management indicators for early warning systems.

Moreover, the implementation of this system provided the necessary support for the deployment of Artificial Intelligence (AI). In the initial stage, the training of the adopted AI model began with qualified personnel from the Northeastern Argentina Fruit Fly Program (PROCEM NEA) identifying and marking fertile specimens of *Ceratitis capitata* in field-captured images, followed by comparisons with determinations made using laboratory instruments. Once a reliable level of adjustment was achieved, implementation began within the PROCEM NEA region, which maintains a surveillance network of over 1,400 active traps distributed across approximately 57,000 hectares of sweet citrus and blueberries. The validation of results between AI and laboratory analysis is continuous; as of January 2026, over 9,000 comparisons have been conducted, yielding accuracy rates exceeding 85% in determinations. This has significantly reduced the turnaround time for obtaining results from Jackson traps and has improved laboratory control processes.

The next objective is to deploy this tool in Pest-Free Areas to expedite the counting of sterile *Ceratitis capitata* specimens, allowing for a dynamic adjustment of the preventive sterile insect release strategy. Given the economic significance of the pest as a limiting factor for fruit exports in Argentina and for domestic trade, strengthening the Surveillance System through IT tools is essential for ensuring efficiency in decision-making and pest management. Consequently, the use of digital field records combined with the AI-powered SIGPROCEM platform represents a qualitative leap in PROCEM's analytical capacity, consolidating it as a fundamental tool for Integrated Pest Management (IPM) by ensuring that strategies remain dynamic and based on solid, precise, and reliable information.

Keywords: Artificial Intelligence (AI), Phytosanitary surveillance, QR.

Area-wide IPM and Sterile Insect Technique: A case study on dragon fruit in Viet Nam.

Nguyen Thi Thanh Hien¹, Dang Dinh Thang¹, Le Thi Xuyen¹, Rui Pereira²

¹Entomology and Nematology Department, Plant Protection Research Institute (PPRI), Viet Nam; ² Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Vienna, Austria. Email: thanhchien1456@gmail.com

Background: Dragon fruit is cultivated intensively in Lam Dong province (formerly Binh Thuan Province), with a total production area of nearly 28,000 ha, of which about 80% is for export. The crop produce fruit year-round, and the average harvest interval ranges from 2.5 months to 3 months. Dragon fruit is adversely affected by *Bactrocera dorsalis* and *Bactrocera correcta* but *B. dorsalis* is a primary pest. By collecting data on fruit fly populations in dragon fruit grown area for 5 years, we implemented an area-wide integrated pest management (AW-IPM) project integrating the sterile insect technique (SIT). The project had two phases: Phase I –Suppression of fruit fly populations by using AW-IPM; Phase II –Integrated suppression with SIT.

Methods: Phase I, we applied three suppression methods for the management area of 1,605 ha: (i) Sanitation including collection and bury infested fruits; (ii) Use of male annihilation technique (MAT) using blocks with methyl eugenol attractant and insecticide; (iii) Spraying a mix solution of protein bait (commercial product: Ento-pro 150 DD) and insecticides (active element: Spinosad). Phase II, we expanded the control area to nearly 2,000 ha. Measures for (i) and (ii) were maintained; while protein bait was sprayed only at the area named “hotspots” where was confirmed as fruit fly production area. Additionally, *B. dorsalis* pupae were irradiated at 90 Gy and released one day before adult emergence. The fruit sampling and the trapping networks were established and implemented to assess suppression performance.

Results: Fruit fly population densities decreased substantially FTD’s average for the period of 2020-2025, which were 6.6 in the AW-IPM & SIT pilot and 21.35 in the control area. The incidence of dragon fruit infestation by fruit fly remained consistently low after the integrated control project was implemented (< 8%).

Conclusions: The integration of SIT technique into the AW-IPM program in fruit fly management is effective and feasible in dragon fruit production areas in Ham Thuan Bac, Lam Dong province.

Key words: *Bactrocera dorsalis*, Oriental fruit fly, area-wide management

Using digital methods for National Fruit Fly Surveillance in South Africa

Julie Mokwele¹, Roedolf Nieuwenhuis², ¹Millicent Mkhize, ¹Jerry Mokwena, ¹Godfrey Makhubela, ¹Emily Munganga & JanHendrik Venter¹

¹Directorate Plant Health, Department of Agriculture, Harvest House Building, 30 Hamilton Street, Arcadia, 0002, South Africa. E-mail: JulieM@nda.gov.za

²BIOSYNTRIX (Pty) Ltd), Komatipoort, 1340, South Africa. Email: roedolf@biosyntrix.co.za

Background: Fruit flies are among the most important agricultural pests, causing direct damage to fruit crops and creating significant barriers to international trade. Effective and continuous surveillance is therefore essential to support early detection, rapid response to outbreaks and informed phytosanitary decision-making. The Department of Agriculture has implemented a national fruit fly monitoring programme since 2006. The Agricultural Pests Act (Act No. 36 of 1963) (APA), is a regulatory framework, for managing fruit fly invasions, together with the South African Emergency Plant Pest Response Plan, and the national *Bactrocera dorsalis* action plan. A digital surveillance system, Biosecurity Africa Application (BAS), developed by Biosyntrix, integrates mobile data capture, cloud-based storage and real-time reporting to support coordinated area-wide fruit fly surveillance

Methods: Real-time field data which includes trap number, lure type, GPS coordinates, trap service dates, and other relevant fields, is collected on BAS. Laboratory diagnostic results can be directly provided on the system once the specimens and all samples are received and identified. The results are directly linked to individual trap records, ensuring full traceability from field collection to confirmed species identification, improving diagnostic traceability and reporting turnaround. This integrated workflow has replaced paper-based data collection, ensuring improved data accuracy and reliability of surveillance information.

Results: Currently, more than 2000 active fruit fly traps are monitored nationally using this digital platform, targeting various fruit fly pests, such as *Bactrocera dorsalis* (Oriental fruit fly), *Bactrocera zonata* (Peach fruit fly), *Zeugodacus cucurbitae* (Melon fly) and others. The digital workflow improves surveillance traceability by linking trap inspections, georeferenced locations, specimen submissions and confirmed diagnostic outcomes into a single national reporting system.

Conclusions: The incorporation of digital surveillance technology has strengthened area-wide fruit fly management by improving data quality, traceability and timely response measures. The tool highlights challenges and opportunities for enhanced pest management. In South Africa's experience, the digital tools can modernize national fruit fly monitoring programs and support preparedness for invasive species. More can be achieved on a regional level through data-sharing, automated alerts and harmonized surveillance, supporting collective management of fruit fly risks.

Key words: Fruit flies, digital tool, surveillance, South Africa

COMPETITION AND DISPLACEMENT BETWEEN *BACTROCERA DORSALIS* AND *BACTROCERA ZONATA* IN MAURITIUS

Patel, Nausheen¹, Sookar, Preaduth², Ollee Aaishah¹

¹Entomology Division, Agricultural Services, Ministry of Agro Industry and Food Security, Mauritius

²Agricultural Services, Ministry of Agro Industry and Food Security, Mauritius

*Corresponding email for first author: npatel@govmu.org/ bnausheen04@gmail.com

A general surveillance programme was initiated in February 2015, and pheromone traps using Methyl eugenol were placed on a grid system 2 km apart using GIS coordinates. Locally modified 1 L plastic containers were used with a cotton wick impregnated with 2 ml of a mixture of pheromone and insecticide in a ratio of 1:1. The traps were serviced on a fortnightly basis, and insects collected and preserved in alcohol were brought to the lab for identification.

Bactrocera dorsalis (Oriental fruit fly) was re-intercepted in October 2015 in the surveillance traps in the Eastern part of the island following its eradication in early 2014's, together with the existing population of *Bactrocera zonata* (Peach fruit fly) whose presence dates back to the 1940's.

Both fruit flies have significant records of fruit infestation, leading to major economic damages ranging from 30 to 100 % depending on the crop season, presence of host plants, and control practices in place.

Monitoring results in 2015 indicate the presence of *Bactrocera zonata* given by an average of 0.2 to 6.0 fly/trap/day. In 2017 both fruit flies were co-habiting with the highest records for *Bactrocera zonata* till May, when *Bactrocera dorsalis* was observed to be dominating in some regions namely in the North West, North East and East (FTD 1.5 Bd, FTD 0.4 Bz). As from July 2017 *Bactrocera dorsalis* started dominating in most regions monitored. By February 2018, *Bactrocera dorsalis* out-competed *Bactrocera zonata*, with a significant population across the island reaching FTD 12.4 in the North West.

As from January 2020, a nil record was observed for the Peach fruit fly in most part of the island, with a persisting population of Oriental fruit fly reaching FTD 16.0 in the South. Later in November 2020 no more *Bactrocera zonata* were retrieved from ME pheromone traps, and the population of *Bactrocera dorsalis* in certain areas exploded reaching 60.0 FTD in summer.

Fruit incubation carried out from 2020 to 2024 showed that *B. dorsalis* displaced *B. zonata*, in mango *Mangifera indica* L., carambola *Averrhoa carambola* L., guava *Psidium guajava* L. and Indian almond *Terminalia catappa* L, with an infestation index as high as 75.64 flies kg⁻¹ fruit, in some preferred hosts.

The results demonstrate the aggressiveness of *Bactrocera dorsalis* and the competitive displacement of *Bactrocera zonata*.

Keywords: fruit fly surveillance, GIS, Pheromone traps, Fly per trap per day, Methyl eugenol

~~**Session 9: Area-wide Integrated Methods Pest Management and Action Programs**~~

DETECTION AND MONITORING OF FRUIT FLY (DIPTERA: TEPHRITIDAE) IN ELSSUKI, SENNAR STATE, SUDAN

Abdelaziz E. Gesmallah, Hamza M. Ahmed and Saifeldeen Y. Elmogadam

The National Institute for Promotion of Horticultural Exports, University of Gezira, Wad Medani, Sudan

Department of Pesticides and Toxicology, Faculty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan

Administration of Plant Protection, Ministry of Agriculture, Sennar State, Sudan

E-mail: azizatniphe@gmail.com

Background: Fruit flies severely damage important fruit and vegetable crops in the Sudan, the production and export of horticultural crops is seriously affected by the fruit flies. Sennar State was selected for carrying out this study because it is the largest fruit producing state in the Sudan.

Methods: Surveys were carried out during June – December 2019 to identify to prevailing fruit fly species in fruits orchard at Elssuki locality, Sennar State. Mcphail traps baited with Trimedlure Terpinyl acetate and cue lure sexual attractants was used to capture to fruit fly specimens. Collection of infested mango, guava and banana fruits were also carried out to provide knowledge about the relation between fruit fly species and their host in this area.

Results: Results of this study showed that both mango and guava fruit were infested by *Bactorocera dorsalis* and *Bactorocera zonata* 66%, 34% and 54%, 46%, respectively. While banana fruits were infested by one species, *Ceratitis cosyra*. Seven fruit fly species: *Dacus celiatus*, *Dacus punctatiformis* (Karash), *C. cosyra*, *C. quinaria*, *C. capitata*, *B. dorsalis* and *Zeodacus cucurbitae* were the usually trapped fruit fly species using the sexual attractants during this study scoring 3, 6, 6, 8, 20, 57 and 7335, respectively.

Conclusion: *B. dorsalis* and *B. zonata* are the dominant fruit fly species attacking mango and guava fruit in Sennar State. Further studies should trace the higher population of *Z. cucurbitae* and its economic importance and to formulate and integrated pest management program to avoid outbreak of any of the recorded fruit fly species in this area.

Key words: Fruit fly- mango, guava, detection Sennar- Sudan.

Population Dynamics of the Olive Fly (*Bactrocera oleae*) in Two Olive Orchard Varieties: Picholine Marocaine and Picholine Languedoc

Aziz Ait Si Mbarek^a, Abderrahim El Keroumi^a, Ismaïl Outoukarte^b, Abdelhi Dihazi^a, Aayah Hammoumi^a, My Ahmed Al Aloui El Fels^a.

^aPharmacology, Neurobiology, Anthropobiology and Environment Laboratory, Faculty of Sciences Semlalia, Cadi Ayyad University, Bd. Prince My Abdellah, B.P. 2390, 40000 Marrakesh, Morocco, E-mail: a.aitsimbarek.ced@uca.ac.ma

^bMultidisciplinary Research Laboratory in Didactics, Education and Training, CRMEF Marrakesh Safi.

Background: The olive fly (*Bactrocera oleae* Rossi 1790) cause substantial economic losses to olive production in the Mediterranean basin. This study aimed to characterize the seasonal population dynamics of *B. oleae* in two cultivars at two contrasting sites in Morocco, and to relate adult trap catches to fruit infestation and maturity in order to identify critical risk periods for integrated pest management.

Methods: *B. oleae* populations were monitored monthly from March 2024 to March 2025 in two olive varieties, picholine marocaine and picholine languedoc at two sites in Morocco's Haouz region (LAATTAOUIA and AMZMIZ). Olive traps baited with 8% ammonium bicarbonate and 25% NaCl covered 2,900 to 15,000 m² per orchard. From August to November 2024, 100-fruit samples per cultivar and site were examined every 15 days to determine fruit infestation rate and a maturity index based on fruit color. A multivariate analysis effects of site, variety, and month on total captures, females, and males.

Results: The analysis revealed a highly significant Month effect ($F(12,37)=4,78$, $p<0,001$), significant variety effect ($F(1,37)=5,09$, $p=0,03$), and marginal site effect for males ($F(1,37)=2,73$, $p=0,107$). Amzmiz yielded 779 individuals (69,5%), and 342 (30,5%) for Laattaouia. Picholine marocaine attracted 878 flies (78,3%), and 243 (21,7%) for Picholine languedoc. Peak activity occurred in December 2024 (403 Amzmiz, 201 in Laattaouia). In 1121 total flies captured, males predominated (660). Fruit infestation rose from 3% with a maturity index <0.5 in September to 87% with an index >3.4 in November, paralleling the rise in adult catches at Amzmiz site.

Conclusions: *B. oleae* populations are strongly structured by cultivar, site and season, with a clear preference for picholine marocaine and higher pressure at Amzmiz. Linking adult dynamics with fruit infestation and maturity identifies October and December as a critical risk period. These data may help optimize the timing and intensity of phytosanitary interventions and develop differentiated management strategies according to local context.

Keywords: *Bactrocera oleae*, population dynamics, olive fly, picholine marocaine, picholine languedoc, olive.

Session: Area-wide Integrated Pest Management and Action Programs.

Planning for resistance: USDA APHIS efforts to prevent and mitigate spinosad resistance in regulated fruit fly species intercepted in the United States.

Dara G. Stockton, Ikkei Shikano, Mamoudou Setamou

United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine, Science and Technology, Insect Management and Molecular Diagnostics Laboratory (IMMDL), 22675 N Moorefield Road, Edinburg, Texas, 78541, USA. E-mail: dara.stockton@usda.gov.

Background: Insecticide resistance among invasive tephritid fruit flies poses a greater-than-normal risk to national food security and has the potential to disrupt quarantine and eradication programs, which rely on the efficacy of Spinosad as a major component of IPM-based programs to prevent widespread establishment in North America. USDA APHIS administers a combination of attract-and-kill treatments, ground treatments, and foliar applications of insecticides in affected areas during quarantine and eradication programs. Due to the recurring incidence of spinosad resistance in *Bactrocera* spp. and *Zeugodacus* spp. in several East Asian countries and Hawaii, where several fruit fly species are established, APHIS has invested in research to identify spinosad alternatives that meet reduced-risk pesticide criteria and develop methods of resistance detection and mitigation methods.

Methods: During 2021, USDA and the University of Hawaii completed widespread resistance survey work to identify the extent of resistance across the inhabited portion of the archipelago in *Zeugodacus cucurbitae* and *Bactrocera dorsalis*. Three resistant lines of *Z. cucurbitae* collected and maintained in colonies in Hawaii were used to test the comparative efficacy of various Spinosad alternative insecticides mixed with Nu-Lure bait solution, and a vial assay was developed to quickly screen for resistance in the field using live caught fly samples. In Texas, the USDA and University of Texas A&M Kingsville identified parallel insecticides that could function as spinosad alternatives against *Anastrepha ludens*, which is an annual incursion pest along the Texas-Mexico border.

Results: Bioassay work with spinosad-resistant lines in Hawaii identified insecticides with greater risk for cross-resistance, as well as those that remained effective against resistant *Z. cucurbitae*. In Texas, at least two additional insecticides were found effective in an ingestion-based method against *A. ludens* and appropriate for formulation in wax-bait stations. For the vial assay, we identified a diagnostic dose of spinosad effective at killing 100% of susceptible flies within 4 h of exposure and produced a predictable response curve according to the resistant ratio of the tested population when used against resistant lines. The vials were also found to be shelf stable for up to 6 months.

Conclusions: Trials to test the effective insecticides in formulation with existing attract-and-kill devices are ongoing but are expected to produce a short list of recommended options that can be integrated into a rotation-style approach, augmenting the spinosad-only bait sprays and wax bait stations currently employed by APHIS. Detection of resistance remains an area of concern, particularly in regions where live detections are scarce, but the vial assay remains an important tool for gathering samples in the field to screen for genetic markers that may eventually be used for genetic detection when intercepted samples are submitted for identification. Further, in states like Hawaii that

have established populations, the vial assay can serve as a valuable tool for efficient and affordable resistance monitoring by growers, states, and federal agencies as needed.

Key words: *Bactrocera*, *Zeugodacus*, *Anastrepha*, spinosad, insecticide resistance

**Preliminary survey for the Oriental fruit fly, *Bactrocera dorsalis*
(Diptera, Tephritidae) in northwestern Libya**

Esam Elghadi¹ , Khdiya Ali² , Abdusalam Elgattaa¹ , Muhammad Almouzougy³

¹ National Project for Integrated Fruit Fly Management, Libyan Biotechnology Research Center, Libya

² Faculty of Science, Aljufra University, Libya

³ The National Center for Prevention and Agricultural Quarantine, Libya

E-mail: esamelghadi@lbtrc.edu.ly, esam742002@yahoo.co.uk

Background: Invasive fruit fly (Diptera: Tephritidae) species remain the most economically important pests to the agricultural production in the world including the Mediterranean region. With increasing global trade in fruits and vegetables, some polyphagous exotic tephritid species pose a real threat to Libya's food security, especially in the absence of strict quarantine restrictions and lack of inspection points on the country's main entries.

Methods: In this study, an infested mango imported from abroad was detected in a public fruit mini market in the city center of Tripoli in August 2023. The infested fruit was dissected and examined in laboratory. Larvae were detected feeding into the fruit tissue and kept until adult emergence. A preliminary survey was conducted from October 2023 to December 2025 to determine if the alien fruit fly is present in the field. The survey was conducted in nine municipalities by McPhail traps baited with methyl-eugenol. A sampling of infested fruits was performed when and where is possible from some of trapping sites in the chosen municipalities. The identification of the fruit flies obtained from the infested mango, traps and fruits was based on the morphological characteristics of the specimens. For further diagnostic analysis and confirmation, the specimen was forwarded to Invertebrates Unit, Royal Museum for Central Africa, Belgium.

Results: Initial identification was confirmed and further detailed to species level, resulted in identifying the specimen as *Bactrocera dorsalis* (Diptera, Tephritidae). All the adult male specimens collected from the traps belong to *Bactrocera zonata*. Only *Ceratitis capitata*, *B. zonata* and *Dacus frontalis* flies were yielded from fruit sampling in the survey sites.

Conclusions: This is the first record of *B. dorsalis* introduced intercepted in Libya. The pest status of *B. dorsalis* is absent and intercepted only. Early detection surveillance should be carried on. Awareness of exotic invasive fruit fly pests and improvements of quarantines for such species should be raised

Key words: Mango; Invasive species; fruit flies; *Bactrocera dorsalis*; Libya.

Modelling-Informed Decision-Making in Guatemala's Medfly Program

Estuardo Lira, Alene Seiler-Martinez, Gwen Keller[†]

U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS),
Action Programs, Medfly Program (Guatemala, Mexico, Belize), U.S. Embassy, Blvd. Austriaco
11-51, Guatemala City, GT, 01016. e-mail: estuardo.lira@usda.gov

Background: The Mediterranean fruit fly (*Ceratitidis capitata* (Wied.)) has posed a persistent threat to horticultural production, trade, and food security in the US, Mexico and Guatemala since its detection in the region. The Medfly Program officially began operations in Guatemala in 1975, establishing a national surveillance trapping network to monitor pest distribution and population dynamics. In 2004, the Program incorporated Geographic Information Systems (GIS) to georeference trap locations and integrate environmental variables, including temperature, rainfall, soil texture, and coffee-growing areas. Data generated between 2004 and 2007 supported the implementation of the Gradual Advance Plan (GAP), a cost-effective eradication strategy, and enabled early analyses linking trap captures with the El Niño–Southern Oscillation (ENSO). However, during 2019–2020, Medfly populations reached historically high levels, resulting from a convergence of favourable climatic conditions and strategic decisions that were later reassessed.

Methods: Species Distribution Modelling (SDM) techniques were applied using Medfly surveillance data collected between 2004 and 2020. Generalized Linear Models (GLMs) were developed in R to evaluate the relationship between Medfly abundance and environmental drivers, as well as the mitigating effects of two primary control interventions: the Sterile Insect Technique (SIT) and bait spray applications. Model outputs were translated into spatial risk maps depicting the probability of Medfly occurrence across habitats and altitudinal gradients in Guatemala. In parallel, population trends were analysed using analogue years to forecast potential population trajectories under varying climatic and operational scenarios.

Results: The modelling framework clarified the relative influence of environmental conditions and control interventions on Medfly population dynamics. While higher temperatures were associated with increased population pressure, sustained area-wide application of the Sterile Insect Technique (SIT), complemented by timely bait sprays, effectively suppressed populations. The use of predictive modelling supported targeted deployment of control measures, improved resource allocation, and enhanced the program's capacity to respond to climatic variability within an area-wide integrated pest management framework.

Conclusions: Integration of long-term surveillance data with species distribution modelling strengthened strategic planning and operational decision-making within the Medfly Program. Model outputs informed strategic adjustments, supported funding decisions, and guided operational prioritization, contributing to the reversal of the 2019–2020 outbreak and the reduction of Medfly populations to levels comparable to 2015 by 2025.

Key words: *Ceratitidis capitata*, Species Distribution Modelling, Sterile Insect Technique, Integrated Pest Management

MASS REARING, QUALITY CONTROL, AND STERILIZATION OF DROSOPHILA SUZUKII IN THE SANTA ROSA BIOPLANT (ISCAMEN)

Juan Asfennato, Estela Tur, Rolando Martinez, Asfennato Sol, Jaquelina Tur, Erica Gómez Barrionuevo, José Orts

Institute of Agricultural Health and Quality of Mendoza (ISCAMEN), Mendoza, Argentina.
E-mail: aleasfennato@yahoo.com.ar

Background: A bisexual colony of *Drosophila suzukii* with 85 generations of inbreeding is currently maintained and structured into two management levels: a filter colony focused on genetic and morphological selection, and an amplification colony dedicated to mass production. The objective is to sustain production and quality parameters compatible with sterile insect production and field application programs.

Methods: The filter colony operates with two Plexiglas cages containing 30 cc of pupae (approximately 6,000 individuals), where manual selection under stereomicroscope is conducted to confirm species identity, body size, and morphological integrity. Egg collection is performed through direct exposure of larval diet to adults for 24 hours, followed by incubation at 22 °C for 7–9 days under controlled humidity (75–85%). Larvae are recovered using the standard larval diet dissolution method, pupae are separated, dried on absorbent paper, and transferred to the amplification colony. The production colony uses 1DS-MAS cages with a capacity of 250 cc of pupae per cycle and a total production of 1,250 cc. The DS-2 larval diet formulation includes sugar, torula yeast, carrot powder, dehydrated potato puree, soybean flour, preservatives, acidifiers, and water. Sterilization is performed using the IMCO-20 irradiator at Santa Rosa Bioplant, applying a total dose of 220 Gy delivered in two passes to ensure homogeneous irradiation.

Results: Average weekly production reaches approximately 100 cc of pupae, equivalent to an estimated 1 million pupae per week. Quality parameters include a mean pupal weight of 1.68 mg, egg viability of 85–95%, adult emergence of 72.5%, proportion of fliers of 71.1%, sterility levels ranging from 95.5% to 99.5%, approximately 220,000 pupae per liter, and an estimated yield of 40 million pupae per ton of diet. Quality parameters have remained stable; however, adult emergence is the most environmentally sensitive parameter, particularly under low relative humidity. Weekly shipments of sterile pupae to Patagonia have been conducted, with transport times of 24–30 hours from irradiation to box opening at destination. Preparations for shipments to Chile are ongoing pending regulatory authorization.

Conclusions: The mass-rearing, selection, production, and sterilization system has been optimized and standardized, allowing the maintenance of stable and reproducible production and quality parameters and ensuring a reliable supply of sterile pupae for logistical evaluation and field applications. The refinement of the rearing protocol supports scalable production according to operational demand, providing flexibility for future program expansion. Humidity control during larval and pupal stages was identified as a critical factor for improving adult emergence.

Key words: mass rearing; *Drosophila suzukii*; Sterile Insect Technique; quality control; irradiation; pupal production;

Session 9. Sterile Insect Technique

AREA-WIDE INTEGRATED PEST MANAGEMENT (AW-IPM) OF MEDITERRANEAN FRUIT FLY IN MENDOZA, ARGENTINA

Juan Asfennato, Érica Gómez Barrionuevo, Mauricio Páez, Romina Quiroga, Beatriz Turinetto, José Orts

Institute of Agricultural Health and Quality of Mendoza (ISCAMEN), Mendoza, Argentina.

E-mail: aleasfennato@yahoo.com.ar

Background: The Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) is one of the most economically important quarantine pests affecting fruit production and international trade in Argentina. In Mendoza Province, the Mendoza Institute of Agricultural Health and Quality (ISCAMEN) implements an Area-Wide Integrated Pest Management (AW-IPM) program with a strong Sterile Insect Technique (SIT) component aimed at preventing establishment and eradicating pest populations across the main productive oases of the province.

Methods: A permanent monitoring system is maintained through a network of approximately 4,000 traps deployed according to phytosanitary risk and land use (urban, peri-urban, and productive areas), following ISPM standards and covering an estimated area of 600,000 hectares. Trapping data are used to assess population dynamics and support operational decision-making. In high-risk zones, mass trapping is applied as a complementary tool, particularly in urban and peri-urban environments with a high density of backyard host plants. The SIT component is based on the continuous production and release of sterile male *Ceratitis capitata* at the Santa Rosa Bioplant through aerial and ground releases. The program also integrates augmentative biological control through releases of the parasitoid *Diachasmimorpha longicaudata*, sanitary harvests in private households to remove host fruits, and outreach and training activities for producers and the general community.

Results: The integrated implementation of surveillance, population suppression, SIT releases, biological control, mass trapping, and community participation has contributed to reducing pest pressure and limiting the risk of establishment and spread of *Ceratitis capitata* in Mendoza.

Conclusions: Sustained application of this AW-IPM strategy has strengthened Mendoza's phytosanitary status and supported the protection of regional fruit production and export markets.

Key words: Sterile Insect Technique; *Ceratitis capitata*; biological control; mass trapping;

OPERATIONAL AND ECONOMIC VALIDATION OF REAL-TIME IOT-BASED MONITORING SYSTEMS FOR ZERO-TOLERANCE COMPLIANCE AGAINST *CERATITIS CAPITATA* IN MOROCCAN CITRUS ORCHARDS

Mohamed Darkaoui¹, Tahar Hamdani², Amine Leghlid³, Abdelaziz Zahidi^{1,4} and

Said Aminzou¹

¹: Applied Entomology Laboratory, Agadir Horticultural Complex, Hassan II Institute of Agronomy and Veterinary Medicine, Agadir, Morocco

²: PCS AGRI, Agadir, Morocco

³: Sterile Male Production Center, Ait Melloul, Morocco

⁴: Laboratory of Biotechnology and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco

E-mail : mohamed.darkaoui@iav.ac.ma

Background: In the Souss-Massa citrus production area, compliance with Zero Tolerance phytosanitary export protocols places immense pressure on fruit fly monitoring. Standard manual scouting, while functional, suffers from decision-making latency ("Time-Lag") and increasing operational drudgery. While Internet of Things (IoT)-based automation offers a technical solution for real-time surveillance, its high initial acquisition cost remains a major barrier to adoption. This study aims to validate the technical performance of a smart trapping system (CapTrap) while demonstrating its medium-term economic viability compared to conventional monitoring strategies.

Methods: A feasibility study was conducted in a commercial orchard to evaluate a network of autonomous smart traps integrating computer vision and Deep Learning algorithms. The protocol focused on two axes:

(i) Technical Validation, focusing on hardware robustness and AI counting accuracy under semi-arid conditions (heat/dust); and

(ii) Economic assessment, based on Total Cost of Ownership (TCO) analysis over 3 years, comparing the high initial investment (CAPEX) of the IoT solution with the recurring operational costs (OPEX) associated with manual monitoring and chemical treatments.

Results: Field trials confirm the technological rigor of the system, which successfully eliminates scouting drudgery and provides real-time flight peak alerts. Financially, the analysis demonstrates that while the entry cost is higher, the investment is amortized over a 3-year cycle. This Return on Investment is primarily driven by a substantial reduction in labor and travel costs associated with monitoring, as well as a measurable decrease in the Treatment Frequency Index (TFI) achieved through preventive targeting rather than systematic calendar spraying.

Conclusion: This study validates the shift towards digitized Precision IPM in citrus orchards. It proves that the initial technological premium is a strategic investment, fully compensated by secured export certifications and the sustainable optimization of phytosanitary inputs.

Key words: *Ceratitis capitata*, IoT, Deep Learning, Smart Trapping, Cost-Benefit Analysis, Amortization, Treatment Frequency Index, Precision Agriculture.

BIODIVERSITY AND INTEGRATED MANAGEMENT OF FRUIT FLIES ON FRUIT CROPS IN INDIA

SANDEEP SINGH* & RAJWINDER KAUR SANDHU**

*Principal Entomologist (Fruits)

**Entomologist (Fruits)

Department of Fruit Science, Punjab Agricultural University, Ludhiana, Punjab, India 141004

Corresponding author e-mail: sandeep_pau.1974@pau.edu

Background: Fruit flies are of great economic importance and ruin more than 400 different fruit and vegetable crops. reported 335 species in India belonging to 97 genera in 22 tribes and 5 subfamilies, most important being *Bactrocera dorsalis* (Hendel), *B. zonata* (Saunders), *B. correcta* (Bezzi); *Zeugodacus cucurbitae* (Coquillett) and *jujube* fruit fly, *Carpomyia vesuviana* Costa. These fruit flies are also very difficult to manage due to the fact that they are polyphagous, multivoltine, adults have high mobility and fecundity and all the development stages are unexposed.

Methods: Due to progressively more stringent restrictions on the use of insecticides and the increasing demand for healthy food around the world, new environmentally friendly techniques for fruit fly management are arising and more than one tactic is frequently required.

Results: Male Annihilation Technique (MAT) is the most important component of IPM in India for monitoring and management. In Punjab, the adoption of PAU Fruit Fly Trap is a success story with adoption rate around 90 %. Hot water treatment for export of mango from India is a widely used technique. The use of Sterile Insect Technique (SIT) for management of fruit flies in India is still in beginning stage. Among biocontrol agents, *Diachasmimorpha longicaudata* (Ashmead) is the promising biocontrol agent. Recently, use of RNAi technique has been initiated in India for management of fruit flies in guava.

Conclusions: Fruit flies remain a major threat to fruit production in India.

Key Words: Fruit flies, Fruits, IPM, Biocontrol agents, RNAi

ABSTRACT

Contribution to fruit flies (*Bactrocera dorsalis*) IPM strategy in Bimodal Rainforest agroecological zone in Cameroon

Colince Nguelo, Anne Ceres Nsongo & LIYEMBEME Eone Fatimatou

Sub department of Phytosanitary Intervention, Ministry of Agriculture and Rural Development, P.O Box 2201, Messa, Yaounde, Cameroon. Email: nguelo12cm@gmail.com

Background: *Bactrocera dorsalis* is a dominant fruit fly in Cameroon and responsible for serious constraints to mangoes and other fruit production. Today, mangoes (*Mangifera indica*) play important role in increasing foreign exchange and local food security. The export of mangoes has encountered more than 140 times interceptions at the gates of European Union from 2011 to 2018, due to this pest and resulting in substantial losses. The IPM diversity of tactics has not been available to farmers and the pest control is mostly the use of chemicals and today some pheromones. The lack of knowledge on the distribution and behavior of this pests lead farmers to the misuse of IPM tactics. That is why, farmers have abandoned their farms for fruits export in these 3 localities. More informations are needed to fuel and improve the IPM tactic.

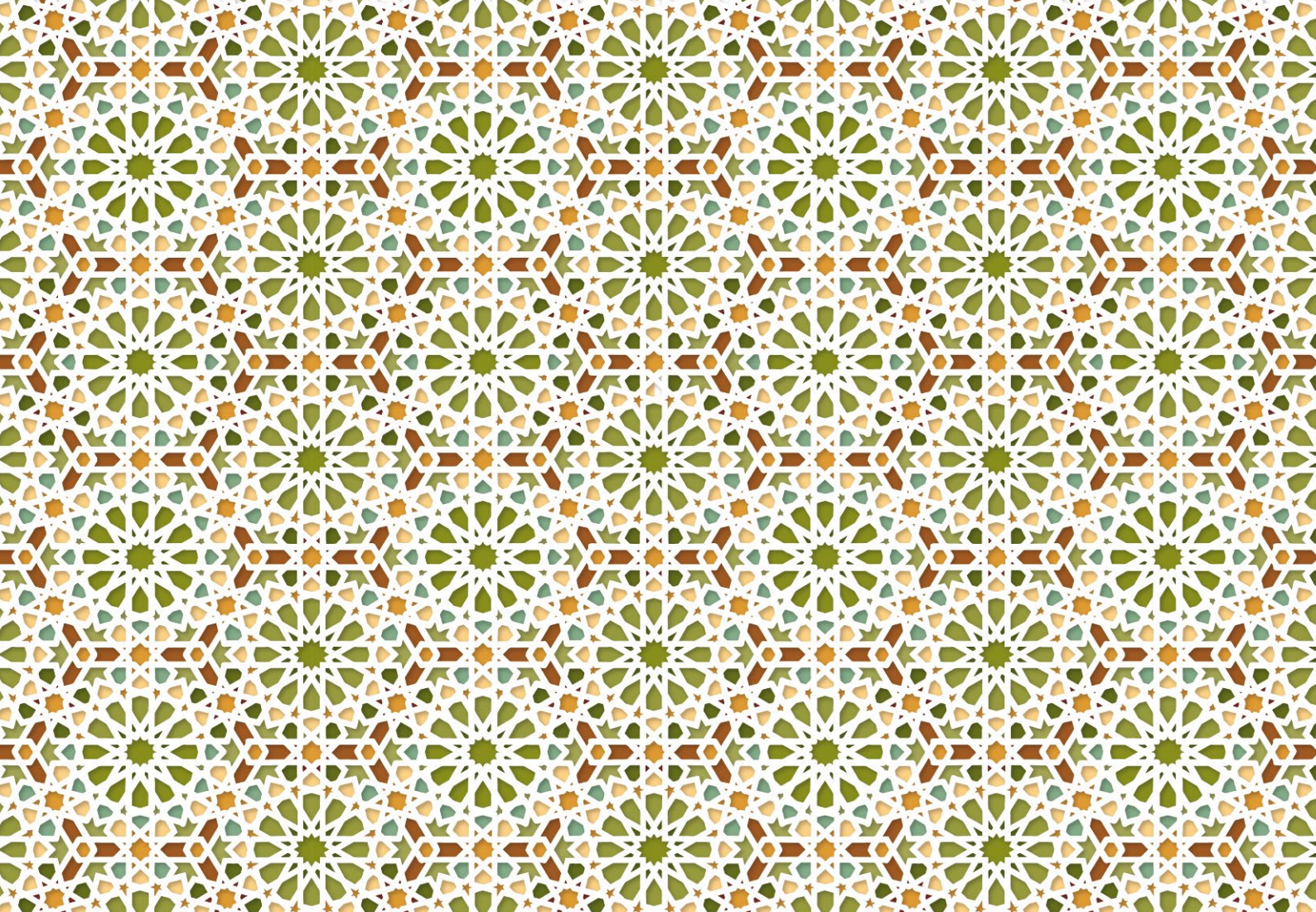
Methods: A Tephri trap was used to monitor the pest in 3 sites, especially where the farms for fruit export were abandoned. One para pheromone and insecticide stick with Dichlorvos was used in 3 traps (with ME) set up in 3 different farms per site. The GPS was used to collect geographical coordinates of each traps and fruit flies were counted every 2 weeks from each trap and during 12 months of 2024. The softwares QGIS/GIMP help for mapping. Microsoft support (Excel) was used to calculate the means and the standard deviation to determine a measure of how widely values are dispersed from the average value for each site and each month (January to December). The distances were 60 km (Mfoundi to Mfou) and 110 km (Mfoundi to Eseka).

Results: First of all, the annual count of *Bactrocera dorsalis* was higher in Eseka than in Mfoundi and Mfou. Secondly in Mfoundi (rural area of Yaoundé town), *B. dorsalis* counts were lowest in November (114.75 ± 33.74) and highest in may (3441.20 ± 849.10). For Mfou site/locality, it was lowest in december (61.25 ± 17.49) and highest in june (1877.25 ± 296.47). Eseka locatily has lowest *B. dorsalis* counts in december (63.75 ± 4.82) and highest in may (2091.20 ± 287.14). *Bactrocera dorsalis* exhibited peaks during the rainy season in May in Mfoundi and Mfou, which coincided with the mango season, while it peaked in June in Esseka.

Conclusions: Our study confirmed that host fruit availability and season's variables (rainfall and temperature) are the key factors that determine fluctuations in *B. dorsalis* species abundance across the Humid Forest with Bimodal Rainfall Agro Ecological Zone in Cameroon. The IPM tactics to suppress the pest should not start from January or march/april as found in the field, but should start from Mid-February to june/july. It is very important to consider sanitation from December of each year. The consideration of monitoring in market and other host plants are also important in IPM tactics package.

Key words: *Bactrocera dorsalis*, Methyl Eugenol, IPM tactic

Session 9. Area-wide IPM and Action Programs



POSTERS

Social, Economic and Policy Issues of Action Programs

PIE: A Pest Management Performance Assessment Tool Framework for Holistic Comparison of Pest Management Strategies

Virginia Nichols, Adrian Leach, Jon Knight, Alexandra M. Collins, M. Megan Quinlan

Centre for Environmental Policy, Imperial College London, London, UK

Email: m.quinlan@imperial.ac.uk

Background: Production of crops necessarily requires management of pests and diseases. As the negative externalities of many pest management approaches become more apparent and production costs continue to rise, growers, scientists and policymakers are seeking new approaches that better balance costs and benefits of pest management. Concomitantly, there is a need for tools that can identify practices that balance these goals without burdensome data requirements.

Methods: To meet this need, we united several tools into a pest management performance assessment tool (PESTO) framework that provides a holistic comparison of pest management strategies. PESTO combines a quantitative estimate of potential pesticide impacts using open-source datasets and conversion of these impacts into monetary values, coupled with a qualitative six-component rating system.

Results: While the framework is customizable and can be implemented in a range of computing environments including spreadsheet software or statistical programming languages, we provide an open-source implementation through publicly available Shiny web applications. We demonstrate PESTO's portability between crops and production environments while providing example insights gained from its application.

Conclusions: PESTO provides a holistic, opensource framework that unites quantitative pesticide impact estimates with qualitative assessment, demonstrating portability across crops and production environments. It offers a practical tool for growers, scientists and policymakers seeking to balance the costs and benefits of pest management without burdensome data requirements.

Key words: *pest management, pesticide impact assessment, decision support tool, integrated pest management*

Session: *Social, Economic and Policy Issues of Action Programs*

BIOCONTAINERS: A MEDIATED EDUCATIONAL APPROACH FOR BIODIVERSITY CONSERVATION IN PRIMARY SCHOOLS ADDRESSING THE MEDITERRANEAN FRUIT FLY PROBLEM

William Navarro, Rubén Mellado, Beatriz Turinetto, Alejandro Asfennato
Institute of Agricultural Health and Quality of Mendoza (ISCAMEN), Mendoza,
Argentina.
E-mail: willykabe@hotmail.com

Background: Mendoza is one of Argentina's main agri-food producing regions. Favorable agroclimatic conditions and sustained plant health policies have resulted in a strong phytosanitary status that requires long-term community engagement to be preserved. In this context, the Biocontainers project was developed to promote awareness of plant health protection and the risks posed by established pests such as the Mediterranean fruit fly (*Ceratitis capitata*), as well as potential invasive species.

Methods: The project is implemented through a mediated cardboard box (Biocontainer) containing pupae or cocoons of sterile Mediterranean fruit flies produced at ISCAMEN's Santa Rosa Sterile Insect Bioplant. A viewing window allows students to observe insect development and understand the role of the Sterile Insect Technique (SIT) in protecting agroindustrial production. Each school year, approximately 34,672 primary school students participate in the initiative. The project is jointly implemented by ISCAMEN and the General Directorate of Schools of Mendoza, aligned with the Provincial Curricular Design and the Sustainable Development Goals. Teachers and school authorities from public, private, rural, and special education modalities are trained prior to classroom implementation using a Project-Based Learning approach.

Results: Initiated in 2014 as a pilot experience, the project expanded by 2019 to include all educational modalities throughout the province. It also promoted educational visits to the Santa Rosa Bioplant, which currently receives around 1,500 students annually, and became a strategic component of provincial educational planning. Increased online searches related to ISCAMEN and the Mediterranean fruit fly, as well as growth in institutional website visits, were observed following project implementation.

Conclusions: After ten years of implementation, evaluations involving former participants demonstrated strong long-term knowledge retention, highlighting the effectiveness of experiential learning. Biocontainers constitute a sustainable educational tool that supports environmental awareness, plant health protection, and understanding of SIT-based pest management strategies.

Key words: environmental education; Sterile Insect Technique; Mediterranean fruit fly; plant health; community awareness; Argentina

IMPACTS OF THE “TARIFF HIKE” ON FRUIT FLY POPULATIONS IN THE SÃO FRANCISCO VALLEY, BRAZIL

Itala Cruz Damasceno¹, **Jair Fernandes Virginio¹**

¹Moscamed Brasil, Av. 1, 992 – Distrito Industrial São Francisco, Juazeiro, Bahia, Brazil – ZIP Code 48,909-733. Corresponding author: jair@moscamed.org.br

Background: Fruit flies (Diptera: Tephritidae) represent one of the major phytosanitary constraints to fresh fruit exports, particularly in mango and grape producing regions. In the São Francisco Valley, these crops are of high economic importance and are subject to strict quarantine requirements imposed by importing countries. In 2025, the imposition of additional tariffs by the United States on Brazilian mango exports, hereafter referred to as the “tariff hike,” directly affected the regional production chain, resulting in reduced exports and potentially influencing phytosanitary management practices.

Methods: The study was conducted in commercial mango and grape production areas located in the São Francisco Valley, a region characterized by a semi-arid climate and a strong specialization in irrigated export-oriented fruit production. Fruit fly populations were monitored using trapping systems and expressed as the FTD index (flies per trap per day). Jackson traps baited with Trimedlure were used for the capture *Ceratitis capitata* males. McPhail traps containing 5% hydrolyzed protein diluted in water (500 mL per trap) were used to capture fruit flies of several species. Population monitoring data corresponding to epidemiological weeks 01 to 52 of the years 2024 and 2025 were analyzed. Analytical emphasis was placed on epidemiological weeks 32 to 48 of both years, corresponding to the duration of the tariff measure.

Results: Fruit fly population dynamics differed between the evaluated years. In 2025, the FTD index showed significantly higher values during weeks 32 to 48, ranging from 6.01 to 19.55, whereas in 2024 values remained considerably lower during the same interval. During weeks 01 to 31, FTD values were similar between the two years, indicating comparable population dynamics prior to the trade restrictions. In 2025, FTD values ranged from 3.38 to 19.55, demonstrating high population fluctuation.

Conclusion: Fruit fly populations increased significantly in the São Francisco Valley in 2025, concomitant with the tariff hike on Brazilian mango exports to the United States. The findings support the hypothesis that increased fruit supply to the domestic market resulted in reduced prices. Historically, an inverse relationship exists between fruit prices and the FTD index, indicating that economic factors may indirectly influence phytosanitary conditions.

Keywords: *Ceratitis capitata*; *Anastrepha spp.*; population monitoring; export fruit production; tariffs

PESTFLY PATROL: Early Detection of *Bactrocera dorsalis* in Belgium through citizen science

Luiza Mitrache, Massimiliano Virgilio, Marc De Meyer (RMCA), Jochem Bonte, Wannes Dermauw (ILVO) & Alexandre Kuhn (CRA-W)

Royal Museum of Central Africa (RMCA), Leuvensesteenweg 13, 3080 Tervuren, Belgium. Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Burg. Van Gansberghelaan 96, 9820 Merelbeke. Centre Wallon de Recherches Agronomiques (CRA-W), Bâtiment Alfred Serge Balachowsky, Rue de Liroux 2, 5030 Gembloux
E-mail: luiza.mitrache@africamuseum.be

Background: The oriental fruit fly (*Bactrocera dorsalis*) is one of the most economically damaging fruit fly pests worldwide, and its progressive spread across Europe calls for innovative, scalable monitoring approaches. PESTFLY is a new Belgian initiative integrating structured citizen science to detect the potential arrival or early establishment of *B. dorsalis*. The project is jointly implemented by ILVO, CRA-W and the Royal Museum for Central Africa, with pilot testing in 2025 and the nationwide monitoring campaign *Pestfly Patrol - Who will catch the fly* launching in summer 2026.

Methods: The engagement strategy is built around a phased communication and recruitment pipeline. A preliminary trial in Brussels with seven participants (September 2025) evaluated the usability of the monitoring protocol and iNaturalist workflows. Feedback from participants is essential for adapting the user manual ahead of the nationwide monitoring period, which will involve at least 100 citizen scientists. In early 2026, project communication will be deployed through partner websites, a public landing webpage, and grassroots organizations communication channels. Citizen scientists will receive a standardized monitoring package containing a trap, preservative, sampling materials, a user manual, and visual identification aids. The manual guides participants through trap assembly, differentiation of *B. dorsalis* from *Ceratitis capitata* and other Tephritidae, and the procedures for recording and uploading observations to the online platform iNaturalist.

Results: During the active monitoring period (summer 2026), participants will report their observations through iNaturalist, enabling real-time interaction and data validation. Post-monitoring communication will provide participants with summarized results and contribute to stakeholder awareness. The scientific and social impact of the citizen science monitoring will be evaluated, by addressing the following key aspects: (1) raising public awareness of quarantine species and their impacts, (2) scaling up data collection across larger spatial areas, (3) enabling earlier detection compared to official trapping, and (4) surveying environments that would otherwise be difficult to monitor.

Conclusions: This project demonstrates how citizen science can complement traditional entomological surveillance by expanding spatial coverage, strengthening public engagement and invasive species awareness, and improving early warning capacity for invasive fruit flies.

Key words: *Bactrocera dorsalis*, citizen science, monitoring, engagement, awareness

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