

# **Insect Pest Control Newsletter**



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# **To Our Readers**



Pilot releases of sterile Drosophila suzukii in strawberry greenhouses in Mendoza, Argentina to assess the impact on the wild population. The goal is to test the sterile insect technique (SIT) package that was developed for this species at the Insect Pest Control Laboratory (IPCL). Main developments include radiation biology and mass-rearing methods such as appropriate adult holding cages and egging systems that allow production of a good number of insects in a cost-effective way (Photos: Carlos Caceres, FAO/IAEA).

Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae) is a fruit pest that has recently invaded Europe and the Americas. This species now has a worldwide distribution. Unlike other Drosophilidae that thrive on harvested decaying fruits, the SWD attacks a wide range of soft fruits on the tree with a preference for blueberries, strawberries, raspberries and stone fruits such as cherries, both in open field and in confined production systems such as greenhouses. Therefore, SWD has become a severe threat for the production of soft fruits almost worldwide.



Damaged strawberry due to an infestation by Drosophila suzukii.

As a result of the threat of this new invasive pest species, FAO/IAEA Member States have approached us to assess the possibility of using the sterile insect technique (SIT) against this species. In view of its high reproductive rate and short generational time, it was assumed that SWD was maybe not the best candidate to be controlled using SIT. However, due to its economic importance and the lack of alternatives to insecticide application, we decided that it would be worthwhile to assess the feasibility of using the SIT against this pest in confined environments, such as greenhouses, where the application of chemical insecticides destroys most of the biological control schemes.

Five years ago, we started to develop the SIT package for this pest at the Insect Pest Control Laboratory (IPCL). The research was reinforced by two cost-free PhD students who were hosted at the IPCL under the SUZUKILL project (Managing cold tolerance and quality of mass-produced *Drosophila suzukii* flies to facilitate the application of biocontrol through incompatible and sterile insect techniques') and by research networks within the framework of the ongoing Coordination Research Project ('Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management'), initiated in 2017.

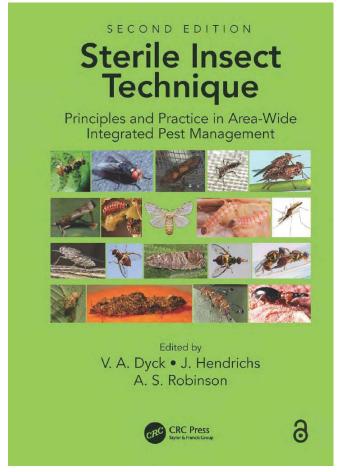
The main progress made relates to the radiation biology with radiation dose responses established, and the development of rearing methods including appropriate adult holding cages, efficient egging systems and suitable larval diets. The feasibility of integrating the SIT with other control methods to manage SWD populations in confined cropping systems is done in collaboration with colleagues from affected countries like Argentina.

A fruitful partnership with the USDA on phytosanitary treatments has been ongoing since 2010. It includes research at the IPCL on the effect of physical and biological factors on the efficacy of these treatments that will support the development of future international standards to facilitate trade for exporting and importing countries. Before fresh fruits and vegetables are shipped across oceans, and borders along international trade routes, they may be treated to eliminate, inactivate or sterilize pests. This highly regulated practice of disinfestation typically involves phytosanitary, or postharvest treatments to stop potential risks of pest introduction and is increasingly done using irradiation.

With respect to field programmes, I would like to highlight two important achievements (see detailed information later in this newsletter). The first is a long-term commitment by the Seychelles National Biosecurity Agency from the Ministry of Fisheries and Agriculture on the prevention of invasive fruit fly species. The recent increase in trade is most likely to lead to the introduction of other non-native invasive fruit fly species from importing countries which pose a serious threat to the local fruit and vegetable production. With the support of the Joint FAO/IAEA Programme, the Member State has created a quarantine infrastructure that allowed the inspection of commodities and early detection in the field of invasive fruit fly species. Since 2000, seven interceptions occurred in fruits and vegetables imported to Seychelles, including Bactrocera dorsalis, Bactrocera zonata; Ceratitis cosyra, Dacus ciliatus and Zeugodacus tau. Thanks to this programme, no establishment has occurred so far. This is especially relevant for the destructive Bactrocera dorsalis that has invaded all the sub-Saharan and Indian Ocean countries with the exception of Seychelles.

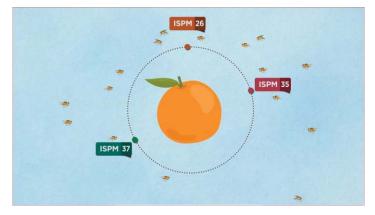
The second achievement is an open field pilot trial conducted by the Cuban Health Ministry to evaluate the feasibility of using the SIT to suppress populations of *Aedes aegypti*. The study was conducted in two isolated neighborhoods located in southwestern Havana. The target area of 'El Cano' has an estimated population of 3 805 residents distributed over 906 houses in an area of 50 ha. For four months, around 40 000 mosquitoes were released twice a week resulting in a 90% reduction in egg hatch. More importantly, no local transmission of *Aedes*-borne diseases has been reported during the last two months of the SIT application in the release area, contrary to the control area where cases were still reported.

A second edition of the book, Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, edited by Dyck V.A., Hendrichs J. and Robinson A.S., will be published soon and available as an open access publication at: (<u>https://www.taylorfrancis.com/</u> <u>books/sterile-insect-technique-dyck-hendrichs-</u> robinson/e/10.1201/9781003035572andhttps://www.routledge.com/Sterile-Insect-Technique-Principles-And-Practice-In-Area-Wide-Integrated/Dyck-Hendrichs-Robinson/p/book/ 9780367474348.



In the second edition, all aspects of the SIT have been updated and the content considerably expanded. A great variety of subjects is covered, from the history of the SIT to improved prospects for its future application. Several chapters discuss the principles and technical components of applying the SIT. The four main strategic options in using SIT (suppression, containment, prevention, and eradication) with examples of each option are described in detail. Other chapters deal with supportive technologies, economic, environmental and management considerations, and the socio-economic impact of AW-IPM programmes that integrate the SIT.

Also, an animated infographic developed in collaboration with the International Plant Protection Convention (IPPC) on 'How to use fruit fly standards to gain market access' was published. Fruit flies present a major challenge to international trade. If they are detected in fruits, trade may be stopped. That is why a set of International Standards for Phytosanitary Measures (ISPMs) on fruit flies has been developed and recently reorganized. When a country wants to export fruit, this set of standards can facilitate the process. Those include ISPM 37 on 'host status'; ISPM 26 on 'fruit fly free areas' and ISPM 35 on 'systems approach'. Through the implementation of these standards, countries protect plant resources from fruit fly pests while, at the same time ensure the application of justified measures that facilitate safe trade. (<u>http://www-naweb.iaea.org/nafa/resources-nafa/multimedia.html</u>).



Another major achievement is the publication of a Special Issue in BMC Genetics (<u>https://bmcgenet.biomedcentral.</u> <u>com/articles/supplements/volume-21-supplement-2</u>) as part of the work conducted during the last five years of the Coordinated Research Project (CRP) on 'Comparing Rearing Efficiency and Competitiveness of Sterile Male Strains Produced by Genetic, Transgenic or Symbiont-based Technologies'.

Two manuals (see details at the Announcement Section of this newsletter), 'Guidelines for Irradiation of Mosquito Pupae in Sterile Insect Technique Programmes' and a second published in English and Spanish on 'Dose Mapping by Scanning Gafchromic Film to Measure the Absorbed Dose of Insects during their Sterilization'. Both can be accessed at the Insect Pest Control website Manuals & Protocols: <u>http://www-naweb.iaea.org/nafa/ipc/public/manuals-ipc.html#mosquitos</u>.

Regarding IPC personnel matters, I am pleased to announce that Ms Maylen Gómez Pacheco was appointed as Entomologist (Livestock and Human Health Pests) to mainly support the transfer of SIT technology to our Member States. Maylen has extensive experience with Mediterranean fruit fly and mosquito operational SIT programmes. She has been a collaborator of the IPC subprogramme for a decade as technical counterpart of TC projects and as chief scientific investigator of research contracts. I give a warm welcome to our new colleague.

I also would like to welcome Mr Robin Guilhot who recently joined the IPCL as a Junior Professional Officer, supported by France. He will be working on the further development of the SIT package for the Spotted Wing Drosophila.

Finally, Mr Rafael Argiles left the IAEA in September 2020. We would like to thank Rafa for his relevant work and contribution to the IPC subprogramme during the last seven years and wish him all the best for the new chapter ahead.

Rui Cardoso Pereira Head, Insect Pest Control Section

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# Forthcoming Events (2021)

### I. Research Coordination Meetings (RCMs) of FAO/IAEA Coordinated Research Projects (CRPs)

Third RCM on Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management. 19–23 April 2021, Seibersdorf, Austria.

Fourth RCM on Improved Field Performance of Sterile Male Lepidoptera to Ensure Success in SIT Programmes. 18 May–21 May 2021, Vienna, Austria.

First RCM on Mosquito Radiation, Sterilization and Quality Control. 31 May–4 June 2021, Seibersdorf, Austria.

Second RCM on Assessment of Simultaneous Application of SIT and MAT to Enhance *Bactrocera* Fruit Fly Management. 28 June–2 July 2021, Vienna, Austria.

Second RCM on Improvement of Colony Management in Insect Mass-rearing for SIT Applications. 30 August–3 September 2021, Vienna, Austria.

Second RCM on Generic Approach for the Development of Genetic Sexing Strains for SIT Applications. 18–22 October 2021, Seibersdorf, Austria.

First RCM Meeting on Improving SIT Fruit Fly Field Programmes. 1–5 November 2021, Vienna, Austria.

### **II. Consultants and Expert Meetings**

FAO/IAEA Consultancy Meeting on Rearing of Lepidoptera for SIT Application. 24–27 May 2021, Vienna, Austria.

FAO/IAEA Consultancy Meeting on Improving SIT Fruit Fly Field Programmes. 7–11 June 2021, Vienna, Austria.

### **III. Other Meetings/Events**

FAO/IAEA Workshop on Design and Evaluation of Mosquito Population Suppression Pilot Trials including Epidemiological Analysis (under Regional TC Project RAS5082). 8–12 February 2020, Nagasaki, Japan.

FAO/IAEA First Coordination Meeting on Enhancing the Capacity to Integrate Sterile Insect Technique in the Effective Management of Invasive *Aedes* Mosquitoes (under Regional TC Project RER5026). 15–19 February 2021, (virtual).

FAO/IAEA Final Coordination Meeting on Enhancing Capacity for Detection, Surveillance and Suppression of Exotic and Established Fruit Fly Species through Integration of Sterile Insect Technique with other Suppression Methods (under Regional TC Project RAF5074). Date and venue to be announced.

Sixteen Session of the Commission on Phytosanitary Measures, International Plant Protection Convention, FAO. 15–19 March 2021, Rome, Italy, (virtual).

XXVI International Congress of Entomology. 18–23 July 2021, Helsinki, Finland.

FAO/IAEA Workshop on Genetic Diversity Analysis and Colony Management. 26–28 August 2021, Seibersdorf, Austria.

11<sup>th</sup> International Symposium on Fruit Flies of Economic Importance. 15–20 May 2022, Sydney, Australia.

# Past Events (2020)

### I. Research Coordination Meetings (RCMs) of FAO/IAEA Coordinated Research Projects (CRPs)

Fourth RCM on Mosquito Handling, Transport, Release and Male Trapping Methods. 14–18 September 2020, (virtual).

### **II. Other Meetings/Events**

FAO/IAEA/WHO Regional Coordination Meeting on the Potential of SIT for the Integrated Control of *Aedes* Invasive Mosquitoes in Europe (under Regional TC Project RER5022). 24–28 February 2020, Athens, Greece.

FAO/IAEA First Regional Coordination Meeting on Assessing the Efficiency of the Sterile Insect Technique for the Control of the Cocoa Pod Borer (under Regional TC Project RAS5086). 9–13 March 2020, Makassar, Indonesia.

FAO/IAEA First Regional Coordination Meeting on Strengthening Food Security Through Efficient Pest Management Schemes Implementing the Sterile Insect Technique as a Control Method (under Regional TC Project RLA5082). The meeting was conducted virtually and split in four sessions on 14, 17 and 24 April and 1 May 2020.

FAO/IAEA Third Coordination Meeting on Strengthening Regional Capacity in Latin America and the Caribbean for Integrated Vector Management Approaches to Control Aedes Mosquitoes as vectors of human pathogens, particularly Zika Virus (under Regional TC Project RLA5074). 13–16 July 2020, (virtual).

FAO/IAEA meeting to finalize the strategic plan titled 'Proposal of a Strategic Plan for the Eradication of the Myasis Caused by the New World Screwworm (*Cochliomyia hominivorax*, Coquerel) in South America and the Subregion Conformed by Uruguay and border areas with Argentina, Brazil and Paraguay' (under Regional TC Project RLA5075). 24–28 August 2020, (virtual).

Fourth Meeting of the Tephritid Workers of Europe, Africa and the Middle East (TEAM). 4–8 October 2020, La Grande-Motte, Montpellier, France, (partially virtual).

FAO/IAEA Regional Training Course on Stakeholder Engagement and Communication Strategy Development for SIT Pilot Projects (under Regional TC Project RLA5074). 19–23 October 2020, (virtual).

Americas Congress on Fruit Flies and the 10<sup>th</sup> Meeting of the Tephritid Workers of the Western Hemisphere (TWWH). 3–7 November 2020, Bogota, Colombia, (virtual).

FAO/IAEA Regional Workshop on Data Analysis and Reporting Methodologies (under Regional TC Project RLA5074). 23–27 November 2020, (virtual).

FAO/IAEA Training Course on Packing, Holding and Release of Sterile Fruit Flies and on Area-wide Fruit Fly Trapping (under National TC Project BOL5022). 7–11 December 2020, (virtual).

# **Technical Cooperation Projects**

The Insect Pest Control Subprogramme currently has technical responsibilities for the following technical cooperation projects that are managed by the IAEA's Department of Technical Cooperation. They can be classed under four major topics, namely:

- Biocontrol using radiation
- Human disease vectors
- Livestock pests
- Plant pests

Country	Project Number	National Projects	Technical Officer
Bolivia	BOL5022	Reducing Fruit Fly Populations in Different Regions Introducing an Integrated Pest Management Approach Including the Use of the Sterile Insect Technique	Walther Enkerlin
Brazil	BRA5061	Using the Sterile Insect Technique to Apply a Local Strain in the Control of <i>Aedes aegypti</i> (Phase II)	Hamidou Maiga
Burkina Faso	BKF5020	Strengthening the Insectarium to Create Agropastoral Areas Permanently Liberated from Tsetse Flies and Trypanosomiasis	Adly Abdalla
Cambodia	KAM5006	Implementing Fruit Fly Surveillance and Control Using Area- wide Integrated Pest Management	Daguang Lu
Chad	CHD5007	Contributing to the Eradication of <i>Glossina fuscipes fuscipes</i> to Improve Food and Nutritional Security	Chantel de Beer
Chile	CHI5051	Implementing Pilot Level of Sterile Insect Technique for Control of <i>Lobesia botrana</i> in Urban Areas	Walther Enkerlin
China	CPR5026	Applying the Sterile Insect Technique as Part of an Area-wide Integrated Pest Management Approach to Control Two Fruit Flies	Daguang Lu
Cuba	CUB5021	Demonstrating the Feasibility of the Sterile Insect Technique in the Control of Vectors and Pests	Rui Cardoso Pereira
Dominican Republic	DOM0006	Building and Strengthening the National Capacities and Provid- ing General Support in Nuclear Science and Technology	Walther Enkerlin
Ecuador	ECU5031	Enhancing the Application of the Sterile Insect Technique as Part of an Integrated Pest Management Approach to Maintain and Expand Fruit Fly Low Prevalence and Free Areas	Walther Enkerlin
Ecuador	ECU5032	Building Capacity for Mass Rearing, Sterilization and Pilot Re- lease of <i>Aedes aegypti</i> and <i>Philornis downsi</i> Males	Maylen Gómez Walther Enkerlin
Ethiopia	ETH5022	Enhancing Livestock and Crop Production through Consolidated and Sustainable Control of Tsetse and Trypanosomosis to Con- tribute to Food Security	Adly Abdalla
Fiji	FIJ5003	Implementing Pesticide-Free Suppression and Management of Fruit Flies for Sustainable Fruit Production	Daguang Lu

Grenada	GRN0001	Building National Capacity through the Applications of Nuclear Technology	Rui Cardoso Pereira
Guatemala	GUA5021	Strengthening National Capabilities for the Control of Agricul- tural Pests Using Nuclear Technologies	Walther Enkerlin
Israel	ISR5021	Assisting in the Development of a Strategy to Counteract Bac- trocera zonata	Walther Enkerlin
Jamaica	JAM5014	Establishing a Self-Contained Gamma Irradiation Facility for the Introduction of Sterile Insect Technique and Experimental Mu- tagenesis and Diagnostic Technologies	Rui Cardoso Pereira
Libya	LIB5014	Supporting Control of Fruit Flies by Establishing a Low Fruit Fly Prevalence Zone	Daguang Lu
Mauritius	MAR5026	Sustaining the Suppression of <i>Aedes albopictus</i> in a Rural Area with Possible Extension to An Urban Dengue-Prone Locality through Integrated Vector Management Strategy	Maylen Gómez
Mexico	MEX5032	Scaling Up the Sterile Insect Technique to Control Dengue Vectors	Kostas Bourtzis
Morocco	MOR5038	Strengthening the Use of the Sterile Insect Technique	Walther Enkerlin Carlos Cáceres
Palau	PLW5003	Facilitating Sustainability and Ensuring Continuity of Area-wide Pest Management — Phase III	Daguang Lu
Senegal	SEN5040	Strengthening National Capacities to Create a Tsetse-Free Zone Using the Sterile Insect Technique	Marc Vreysen
South Africa	SAF5015	Supporting the Control of Nagana in South Africa Using an Ar- ea-wide Integrated Pest Management Approach with a Sterile In- sect Technique Component - Phase I	Marc Vreysen
South Africa	SAF5017	Assessing the Sterile Insect Technique for Malaria Mosquitoes — Phase III	Hanano Yamada
Seychelles	SEY5012	Establishing Area-wide Integrated Pest Management by Using the Sterile Insect Technique in Combination with Other Control Methods on the Suppression of the Melon Fly	Rui Cardoso Pereira
Sudan	SUD5038	Implementing the Sterile Insect Technique for Integrated Control of <i>Anopheles arabiensis</i> , Phase II	Adly Abdalla
Turkey	TUR5026	Conducting a Pilot Program on Integrated Management of Aedes aegypti Including Sterile Insect Technique	Maylen Gómez
United Republic of Tanzania	URT5034	Implementing Pre-Operational Activities for the Elimination of <i>Glossina swynnertoni</i> through Area-wide Integrated Pest Management with a Sterile Insect Technique Component	Chantel de Beer

United Republic of Tanzania	URT5035	Implementing the Sterile Insect Technique as Part of Area-wide Integrated Pest Management for Controlling Invasive Fruit Fly Populations	Daguang Lu
Viet Nam	VIE5021	Integration of the Sterile Insect Technique with Other Suppression Methods for Control of <i>Bactrocera</i> fruit flies in Dragon Fruit Production	Rui Cardoso Pereira

		Regional Projects	
Regional Africa	RAF5074	Enhancing Capacity for Detection, Surveillance and Suppression of Exotic and Established Fruit Fly Species through Integration of Sterile Insect Technique with Other Suppression Methods	Daguang Lu
Regional Africa	RAF5080	Supporting Area-wide Tsetse and Trypanosomosis Management to Improve Livestock Productivity - Phase IV	Maylen Gómez
Regional Asia & the Pacific	RAS5082	Managing and Controlling <i>Aedes</i> Vector Populations Using the Sterile Insect Technique	Marc Vreysen Hamidou Maiga
Regional Asia & the Pacific	RAS5086	Assessing the Efficiency of the Sterile Insect Technique for the Control of the Cocoa Pod Borer	Marc Vreysen
Regional Asia & the Pacific	RAS5090	Advancing and Expanding Area-wide Integrated Management of Invasive Pests, Using Innovative Methodologies Including Atomic Energy Tools	Walther Enkerlin
Regional Europe	RER5026	Enhancing the Capacity to Integrate Sterile Insect Technique in the Effective Management of Invasive Aedes Mosquitoes	Wadaka Mamai
Regional Latin America	RLA5075	Strengthening the Regional Capacities in the Prevention and Progressive Control of Screwworm	Walther Enkerlin
Regional Latin America	RLA5082	Strengthening Food Security through Efficient Pest Management Schemes Implementing the Sterile Insect Technique as a Control Method	Walther Enkerlin
Regional Latin America	RLA5083	Enhancing Capacity for the Use of the Sterile Insect Technique as a Component of Mosquito Control Programs	Maylen Gómez

		Interregional Project	
Interregional	INT5155	Sharing Knowledge on the Sterile Insect and Related Techniques for the Integrated Area-wide Management of Insect Pests and Human Disease Vectors	Rui Cardoso Pereira

### **Highlights of Technical Cooperation Projects**

Establishing Area-wide Integrated Pest Management by Using the Sterile Insect Technique in Combination with Other Control Methods on the Suppression of the Melon Fly (SEY5012)

In Seychelles, two tephritids of economic importance have been established: the Mediterranean fruit fly (*Ceratitis capitata*) and the melon fly (*Zeugodacus curcubitae*). However, increase in trade is most likely to lead to the introduction of other exotic fruit fly species from importing countries which pose a serious threat to local fruit and vegetable production. The main objective of the National Biosecurity Agency (NBA) is to monitor and intervene in order to achieve a zero tolerance with regard to the introduction, establishment and spread of invasive species in Seychelles. This includes promoting and implementing appropriate measures for the control and movement of animals and plants, and their products into and within the country through the enforcement and implementation of Biosecurity legislation and regulations.

The biosecurity officers receive frequent training to boost their capabilities for risk profiling, inspection and verification. In addition, a detection trapping network for invasive species has been installed in the vicinity of the ports of entry with improved equipment, and an increase in fruit sampling and testing is being done to ensure food security of the country.



Fruit inspection at port of entry in Seychelles.

Since 2000, seven interceptions occurred in imported fruits and vegetables by inspection at ports of entry in Seychelles. Those were *Bactrocera zonata* in 2000, *Ceratitis cosyra* and *Bactrocera dorsalis* in mango in 2006, *Dacus ciliatus* in 2010 and 2013, respectively in courgette (squash) and musk melon and *Zeugodacus tau* in 2014 and 2017 in musk melon and tomato. However, the seven interceptions were put immediately under control by implementing the national fruit fly emergency response plan.

The detection trapping network is composed of 33 methyl eugenol traps, 25 cuelure traps and 25 trimedlure traps in addition to the monitoring network with cuelure, trimedlure, and 3 components lure. , In the last 10 years, no captures of invasive fruit fly species were recorded and no exotic fruit fly species were obtained from fruit sampling that is done routinely in Seychelles.



Inspection of detection traps in the vicinity of ports of entry.

The strategies in place to prevent the introduction of invasive fruit fly species such as *Bactocera dorsalis* which is a major threat to the Seychelles should remain a priority. Also, an action plan is in place in case of a detection or interception of adults or infested larvae in the fruits grown in Seychelles. Since 1999, there has been no incursion or possible establishment of any new fruit fly species. This shows that Seychelles has put a great effort to prevent the entry of exotic fruit flies. This is especially relevant for the destructive *Bactrocera dorsalis* that has invaded all of Sub-Saharan and Indian Ocean countries with the exception of Seychelles, causing major economic impact on their fruticulture.

### **Implementing Pilot Level of Sterile Insect Technique for Control of** *Lobesia botrana* in **Urban Areas (CHI5051)**

The European grapevine moth, or *Lobesia botrana* (Lepidoptera:Tortricidae), is an insect pest that develops on more than 200 plant species of various families. It is one of the most serious vineyard pests in the Mediterranean region and southern Europe.

In Chile, the European grapevine moth was detected for the first time in the Linderos area of the Metropolitan Region in April 2008. The pest, which is native to Europe, attacks the vineyards and its larvae cause direct damage by feeding on the grape clusters. This decreases vineyard yields.

In Chile, the pest's potential direct economic damage to the production of table grape, wine, blueberries and plums has been estimated at over USD 75 million per year. In addition, indirect costs are associated with the pest control practices adopted by growers and with the additional costs for the postharvest treatments required to export table grapes.



The European grapevine moth Lobesia botrana (Lepidoptera:Tortricidae).

To face this serious threat, the Agricultural and Livestock Service (Servicio Agrícola y Ganadero, SAG) of the Ministry of Agriculture of Chile declared that it would place the pest under official control. SAG requested support from the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations (FAO) to develop and field test the sterile insect technique (SIT) against the invasive pest.

Since early 2018, the IAEA and the FAO have been engaging in technical cooperation to develop the SIT technology in Chile. This cooperation has included expert missions to Chile, training of technical staff in Canada and South Africa (where the technology is being applied against other moth pests), and supply of key materials and equipment.

The SAG has now established a national *Lobesia* programme. Today, the basic SIT technology package comprises a facility that dedicates 100m<sup>2</sup> to rearing operations, having a production capacity of over 70 000 moths per week. Since October 2018, more than 750 000 sterile moths have been released over a selected suburban infested area where a small-scale *Lobesia* SIT operational programme is being implemented. Field data indicate that the quality of the released sterile moths is good, with 78 percent of the moths being capable of flight and an adequate sterile moth recapture rate. Results are promising and show that the use of a SIT-based integrated pest management approach could lead to the effective suppression of *Lobesia* populations.

This is the first time an area-wide SIT has been developed against this invasive pest species, and the approach is already being used on a small scale to measure the impact on the wild population. Future technical challenges to be addressed in expanding the SIT programme in Chile against the European grapevine moth include the optimization of mass-rearing, as well as replacing ground releases with aerial release using drones.

For more information: FAO leaflet on the sterile insect technique for use against the devastating European grape-vine moth in Chile:

http://www.fao.org/3/ca9542en/ca9542en.pdf.

#### Demonstrating the Feasibility of the Sterile Insect Technique in the Control of Vectors and Pests (CUB5021)

The sterile insect technique (SIT) is a species-specific and environment-friendly method for insect pest control. In most of the countries, the SIT has not reached the operational level for suppressing mosquito vectors of human infectious diseases, with some components requiring further improvement. Despite their remarkable success against agricultural and livestock pests, the decision makers from public health authorities claim scientific evidence of the effectiveness and cost-benefit in controlling mosquitoborne diseases.

The experience with traditional control methods like insecticides has showed that it is not enough to reduce the mosquito populations to impact on the incidence of the diseases. Current methods such as fogging using chemical insecticides, have failed to sustainably control mosquitoes. Therefore, the search of new, innovative, and sustainable alternatives for vector control strategies is necessary. The incidence of dengue is increasing and there is cocirculation of different serotypes in some countries. Zika and chikungunya emerged and spread rapidly worldwide and the yellow fever risk is latent. In the absence of vaccines and efficient drugs to control dengue, chikungunya and Zika virus disease, mosquito control continues to be the most effective way of managing these diseases.



Equipment for the mass-rearing of Aedes aegypti installed at the Institute of Tropical Medicine 'Pedro Kourí'.

Under the framework of the IAEA-TC project and with the support of the Cuban Health Ministry, the local Pan America Health Organization (PAHO/WHO) office, and research institutions, Cuba has started an open field pilot trial to evaluate the feasibility of SIT to suppress populations of *Aedes aegypti*. The SIT pilot project is led by the Institute of Tropical Medicine 'Pedro Kourí' (IPK), and involves several Cuban scientific institutions, such as the Center for Technological Applications and Nuclear Development (CEADEN) and the University of Havana (UH).

From January to April 2020, an open field pilot study was conducted in two isolated neighborhoods located at southwestern Havana. 'El Cano' was selected as SIT intervention site, while 'Arroyo Arenas' in the neighborhood was used as untreated control. 'El Cano' has an estimated population of 3 805 residents and 906 houses in an area of about 50 ha. A social communication campaign, prior to the start of the releases, was developed to inform the local community and request their approval for mosquito releases.

The mosquitos were reared in a small facility located at the IPK. The male insects were sterilized as pupae in a Gammacell 2020 installed at CEADEN.



The first release of mosquitoes in Habana, Cuba was carried out by 'pioneers' from the local school.

A monitoring network with ovitraps was maintained in collaboration with the local populations. Traps were sampled weekly by the health inspectors from the National Program of Vector Control. The dynamics of the wild *Ae. aegypti* population was monitored six-months before and during the release activities. The first release was carried out during a public ceremony with the main stakeholders such as the local government, national health and regulatory authorities and the neighboring residents. It was also attended by a PAHO/WHO representative in Cuba, Dr. Jose Moya Medina. The children from the local school opened the cages to release the insects. Since then, around 40 000 mosquitoes have been released twice a week. After four months of uninterrupted releases, hatching of eggs collected in ovitraps was reduced by 90% as compared with the control area. Also, no local transmission of *Aedes*- borne disease has been reported in the last two months of the SIT application in the release area, contrary to the control area where cases were still reported.



Local designed release-boxes for transport and ground release of mosquitoes.

The pilot study demonstrated the SIT's effectiveness in suppressing wild *Ae. aegypti* populations under urban conditions. All the protocols for mosquito mass-rearing, sex sorting, sterilization, packaging, transporting and releasing were implemented and optimized. In this way, the teams from all institutions involved are ready to scale up the SIT technology to larger areas and with broader goals.

# Coordinated Research Projects (CRPs)

Project Number	Ongoing CRPs	Project Officer
D4.20.16	Comparing Rearing Efficiency and Competitiveness of Sterile Male Strains Produced by Genetic, Transgenic or Symbiont-based Technologies (2015– 2020)	Kostas Bourtzis
D4.40.02	Mosquito Handling, Transport, Release and Male Trapping Methods (2015–2020)	Maylen Gómez
D4.10.26	Improved Field Performance of Sterile Male Lepidoptera to Ensure Success in SIT Programmes (2016–2021)	Marc Vreysen
D4.30.03	Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management (2017–2022)	Carlos Cáceres
D4.20.17	Improvement of Colony Management in Insect Mass-rearing for SIT Applications (2018–2023)	Adly Abd Alla Carlos Cáceres
D4.10.27	Assessment of Simultaneous Application of SIT and MAT to Enhance <i>Bactrocera</i> Fruit Fly Management (2019–2024)	Rui Cardoso Pereira
D4.40.03	Generic Approach for the Development of Genetic Sexing Strains for SIT Applications (2019–2024)	Kostas Bourtzis
D4.40.04	Mosquito Radiation, Sterilization and Quality Control (2020–2025)	Hanano Yamada

### Final RCM on *Mosquito Handling, Transport, Release and Male Trapping Methods.* 14–18 September 2020

The RCM was held virtually and was attended by scientists of 18 participating research institutes from Australia, Brazil, Burkina Faso, China, France, French Polynesia, Indonesia, Italy, Mauritius, Mexico, Philippines, Senegal, South Africa, Spain, United Kingdom and United States of America. The presentations by the participants covered different topics, such as marking procedures, sound and volatile attractants for Aedes, passive traps for Anopheles surveillance, effect of chilling, compacting and transporting at low temperatures on male quality, dispersal, mating competitiveness and survival of Aedes sterile males in the field, ground releases and aerial releases with unmanned vehicles. Given the current travel restrictions imposed by the COVID-19 pandemic, the meeting was held virtually, which required a special effort for the participants to attend from many different time zones around the globe.

Taking advantage of the meeting format, many observers were able to join the virtual presentations. In addition, the participants discussed the plans for the publication of a special issue in a peer-review scientific journal which will consist of scientific papers summarizing the main results and achievements of this CRP.



Participants of the final RCM on Mosquito Handling, Transport, Release and Male Trapping Methods (held virtually).

The main CRP achievements included assessing new traps for mosquito monitoring, the development of large-scale marking techniques, optimization of protocols for handling, packaging and shipping of chilled *Aedes* males, and the development of automatic machines for efficient mosquito release systems. These results complete the SIT package for mosquito and will be very useful in operational mosquito SIT programmes.

#### A Special Issue Published on Comparing Rearing Efficiency and Competitiveness of Sterile Male Strains produced by Genetic, Transgenic or Symbiont-based Technologies

The sterile insect technique (SIT) has been used as a component of area-wide integrated pest management (AW-IPM) programmes to control populations of insect pests and disease vectors worldwide during the last sixty years. The SIT is a species-specific and environment-friendly method, which is based on the mass-rearing, sterilization by ionizing radiation, and release of sterile insects in an area-wide manner to suppress a target population.

Although the SIT has been successful to control pest populations by releasing both sterile males and sterile females, it has been shown with fruit flies that it is more efficient and cost-effective to release only sterile males. In the case of mosquito SIT, it is a prerequisite to release only males given the fact that sterile female mosquitoes blood feed and can potentially transmit major human pathogens such as chikungunya, dengue, Zika, yellow fever among many others.

Male-only releases can be achieved by developing efficient and robust sexing systems. One way to accomplish this is through the development of genetic sexing strains (GSS) in which males and females can be separated, most commonly, based on phenotypic traits such as pupal or eye coloration or on their response to environmental factors such as the temperature. However, the incorporation of such strains in SIT operational programmes depends on their rearing efficiency, genetic stability and mating competitiveness.

A Coordinate Research Project (CRP) on 'Comparing rearing efficiency and competitiveness of sterile male strains produced by genetic, transgenic or symbiont-based technologies' was initiated in 2015 by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. The specific objectives of the CRP were: (1) to comparatively evaluate the performance of sterile males produced by classical genetic, transgenic or symbiont-based technologies; (2) to refine, if necessary, existing technologies and/or adopt new ones for the development and application of strains for the control of agricultural pests and disease vectors and (3) to assess potential genetic instability and/or horizontal (gene) transfer phenomena towards the use of strains developed by transgenic or symbiontbased approaches for SIT applications.

The following summarizes the key achievements and findings of the CRP: (1) an Anastrepha fraterculus sp. 1 GSS

was developed using irradiation and classical geneticsbased approaches using pupal colour as a selectable marker; (2) an Aedes aegypti GSS was developed with an eyecolour selectable marker, and an irradiation-induced inversion for enhanced genetic stability. The strain was introgressed into different genomic backgrounds; (3) novel or refined strains of Ceratitis capitata, Anastrepha ludens, Cochliomyia hominivorax, Aedes aegypti and Aedes albopictus were developed with different technologies; (4) more than thirty strains of different SIT target species were evaluated in respect to their rearing efficiency and mating competitiveness under laboratory rearing conditions, and some of them also under open field conditions (A. ludens, C. hominivorax and Aedes albopictus); (5) no evidence of genetic instability or horizontal gene transfer was observed in the strains tested and, (6) the gene responsible for male sex determination in tephritid fruit flies (C. capitata, Bactrocera dorsalis, Bactrocera oleae, and their related species), maleness-on-the-Y (MoY), was discovered paving the way for novel GSS for SIT target species.



Anastrepha fraterculus *pupal colour-based GSS developed during the Coordinated Research Project. Males emerge from brown pupae while females emerge from black pupae.* 

Eighteen scientists from thirteen countries participated. Major achievements of the CRP were published (19 papers) special issue BMC Genetics in а of (https://bmcgenet.biomedcentral.com/articles/supplements/ volume-21-supplement-2). This special issue provides an important contribution to our knowledge on how sterile male- strains can be produced using different technological platforms and the evaluation of their biological quality in an effort to enhance SIT applications against populations of plant and livestock insect pests and disease vectors.

# Developments at the Insect Pest Control Laboratory (IPCL)

#### **Genetics and Molecular Biology**

#### Temperature sensitive lethal gene and genetic sexing strains

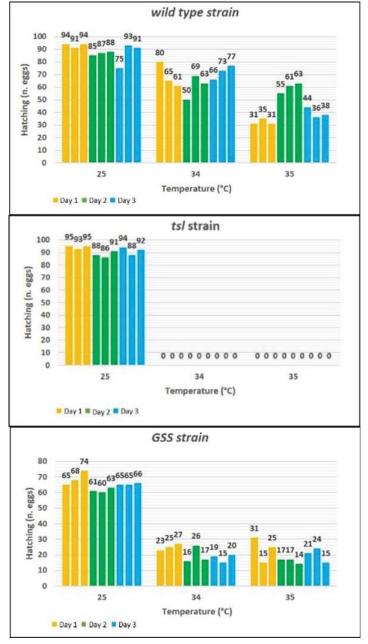
The development and use of genetic sexing strains (GSS) has played a catalytic role in enhancing the efficiency, efficacy and cost-effectiveness of the sterile insect technique (SIT) for the population suppression of insect pest species of agricultural importance. The best examples are the large-scale operational SIT programmes against the Mediterranean fruit fly, *Ceratitis capitata*, in which over 2 billion sterile males of the VIENNA 7 and VIENNA 8 strains are being produced weekly in mass-rearing facilities worldwide.

The VIENNA 8 strain carry two key selectable markers, the *white pupae* (*wp*) and the *temperature sensitive lethal* (*tsl*) genes. Females are homozygous for the mutant alleles of these genes, being white pupae and sensitive when exposed as one-day old embryos at elevated temperatures (34-35 °C) (lethal). Males are heterozygous for both markers, being brown pupae and resistant when exposed as one-day old embryos to the same elevated temperatures (survive).

There are ongoing efforts at the IPCL, and in the frame of the CRP project D44003 on 'Generic approach for the development of genetic sexing strains for SIT applications', to develop a generic approach for the construction of GSS against insect pests and disease vectors. A key factor for the development of a generic approach is the identification of genes used as selectable markers in different GSS such as the *white pupae (wp)*, *black pupae (bp)*, *red eye (re)* and the *temperature sensitive lethal (tsl)* gene.

The presence of the *tsl* mutation in strains of the Mediterranean fruit fly is confirmed by using the so called '*tsl* test' which is based on the exposure of 24 h-old embryos (three replicates of 100 embryos each collected over three consecutive days) for 24 hours at a control (25 °C) and five selected elevated temperatures, i.e. from 31 to 35 °C. Our efforts to identify and characterize the *tsl* gene required the identification of robust reference strains, resistant and sensitive to elevated temperatures, respectively. In this research, we applied a short version of the '*tsl* test' to more than 40 strains of the Mediterranean fruit fly maintained at the IPCL by testing their resistance/sensitivity at two high temperatures, 34 °C and 35 °C. During this test, the hatch rate was recorded as well as the number and colour of pupae, and the emergence of adults (both males and females).

The application of the short '*tsl* test' revealed high variability between the strains tested. However, 'typical' patterns were observed among the 'wild type', '*tsl*' and 'GSS' strains as shown in the figure. In addition, we were able to detect high temperature resistant and high temperature sensitive strains which will be used as reference (control) strains in our efforts to identify and characterize *tsl* gene in Mediterranean fruit fly and next to see if orthologs of this gene are present in other insect pest species which can be used as selectable markers for the construction of GSS similar to the VIENNA 8.



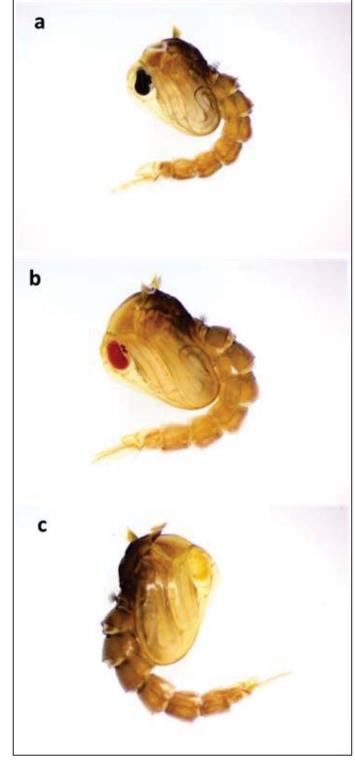
Graphs indicating the application of the short 'tsl test' to a wild type strain (top), a double homozygous mutant wp tsl strain (middle) and a wp tsl-based GSS strain (bottom) by exposing three replicates of each 100 eggs (during three consecutive days) at the control temperature (25 °C) and two elevated temperatures (34 °C and 35 °C). The three days are indicated with different colours: day 1 (yellow), day 2 (green) and day 3 (blue).

#### Construction of *Aedes aegypti* genetic sexing strains and improvement of their stability for SIT applications

Aedes aegypti has been enlisted as one of the major transmitters of arboviruses like Zika, dengue, chikungunya and yellow fever. Current control methods rely on insecticide applications and removal of breeding sites, but these approaches have often failed to reduce Ae. aegypti populations efficiently and sustainably. The well-established SIT can be applied as part of an area wide population suppression strategy to control mosquito populations. One of the most pivotal factors that can increase effectiveness of a SIT programme is a sex separation system that will allow for maleonly releases. An efficient sex separation system will accurately separate males from females and drastically reduce the risk of releasing females. Ae. aegypti presents an evident sexual dimorphism at the pupal stage with females being larger in size than males. Using mechanical tools Ae. aegypti can be sex separated based on this trait, but the efficiency of this technique is highly variable since it depends to a great extent on the rearing conditions.

The development of a classical GSS requires two basic components: a visible or conditional lethal mutation that can be used as a selectable marker and its linkage (wild-type allele) to the male determining locus. In such a case, the males are heterozygous with a normal 'wild-type' phenotype while the females are homozygous for the recessive allele of the selectable marker, expressing the mutant phenotype, and can be separated from males. *Ae. aegypti* males are characterized by the dominant male-determining locus (M-locus) that resides on chromosome 1 and defines male development.

The IPCL has constructed two genetic sexing strains (GSS) for Ae. aegypti using classical genetic approaches. Two eye colour genes, red eve (re) and white eve (w), are located in chromosome 1 and the inheritance of both mutant phenotypes is controlled by sex-linked, recessive genes. Two different strains, red-eye GSS and white-eye GSS were constructed, in which the males are heterozygous with wildtype eyes and females are homozygous with mutant eyes (red or white) (see figure). The recombination frequency in males proved to be similar to the frequency in females, and therefore a filter rearing system was developed based on sorting at four different levels. Combining different sexspecific or sex-linked characters at different stages the recombinant progeny was removed in each generation and allowed the maintenance of pure red-eye GSS and whiteeye GSS colonies (Koskinioti et al. 2020. Genetic sexing strains for the population suppression of the mosquito vector Aedes aegypti. Philosophical Transactions B Royal Society: doi:10.1098/rstb.2019.0808).



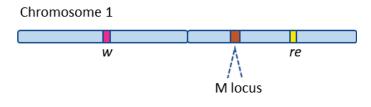
Red-eye GSS and white-eye GSS of Aedes acgypti. a) Black eye male pupa, b) Red eye female pupa, c) White eye female pupa. The males in both strains are heterozygous with wild-type eyes (black eye phenotype) and the females are homozygous with mutant eyes (red or white eye phenotype). Photos credit: Lucia Duran de la Fuente FAO/IAEA.

The quality control analysis of the two GSSs involved evaluation of the sex ratio, the immature development time, productivity and lifespan. Although there were no significant differences in sex ratio and immature development period, the data for productivity and lifespan clearly showed that the red-eye GSS is more prominent and more genetically stable than the white-eye GSS. Two of the most critical aspects for a successful SIT application are the flight ability and the mating competitiveness of the released sterile males. In order to evaluate both factors in the two GSSs, a proper irradiation dose had to be selected beforehand that would be used as the reference value for all downstream experiments. Therefore, an irradiation dose-response curve was developed to determine the optimal dose for induced sterility. Using the selected dose of 90 Gy, the irradiated red-eye GSS performed better than the irradiated white-eye GSS and the control strain regarding flight ability and similar to the control strain regarding male mating competitiveness under laboratory conditions. Accordingly, the evaluation of production parameters and mating competitiveness of the irradiated males ranked the red-eye GSS higher on the list of key performance indicators required for a successful GSS.

Encouraged by the great potential of the red-eye GSS, we tested the ability of the irradiated males to suppress a target population in laboratory cage experiments. Two suppression cages and one fertile control cage were set up and biweekly releases of irradiated males proved to be sufficient to fully suppress a target laboratory cage population.

The development of any GSS also requires genetic stability which is achieved either by the lack of recombination events in males (a common phenomenon in many Diptera) or by chromosomal inversions that can alter the recombination frequencies or suppress recombination events. In *Ae. aegypti* recombination frequency in males is not suppressed and the genetic stability of a GSS faces the threat of the quick accumulation of recombinants and eventual collapse of the GSS. Inversions that occur either naturally in the genome or are induced by irradiation can assist in the genetic stability of GSSs.

Our initial efforts to stabilize the two GSSs were based on the identification of naturally occurring recombination suppressors between the M locus and the mutated loci that lead to the red and white eye phenotypes (re-M and the w-M, respectively) of *Ae. aegypti* (see figure). Initial crosses involved males from several wild type strains crossed with homozygous *re* or *w* mutant virgin females 'en masse' and recombination frequencies were recorded until generation F3. Varying levels of recombination were observed, but none of the combinations could severely reduce recombination, thus urging us to focus on alternatives.



*M locus, red eye* (re) *and white eye* (w) *loci on chromosome 1 of* Aedes aegypti.

Thereafter we used irradiation to induce inversions on the chromosome 1 of a wild type *Ae. aegypti* strain and checked the recombination rates. Screening of several lines resulted in the isolation of inversion lines that could significantly suppress recombination both in the re-M and the w-M regions. These inversion lines could be maintained in the laboratory under normal rearing procedures, suggesting that the chromosomal rearrangements involved did not negatively impact the fitness of *Ae. aegypti*.

One of the inversion lines was incorporated in the red-eye GSS and white-eye GSS and massive screening for consecutive generations of the eye phenotype and its linkage to the sex demonstrated extremely reduced recombination frequencies compared to the original GSSs. The biological quality of the red-eye GSS with and without the inversion was evaluated and indicated that the inversion has a fitness cost and reduces productivity and male flight ability both with and without irradiation.

However, in a cage population suppression experiment the inversion line of the red-eye GSS managed to fully suppress the targeted laboratory population similarly to the original red-eye GSS, thus further promoting our results for the incorporation of an *Ae. aegypti* GSS in a SIT application. The introduction of the irradiation-induced inversion in the red-eye GSS strain has significantly reduced the probability of female contamination in the male release batches and all the above data converge to the conclusion that the irradiated red-eye GSS males can be used in SIT applications to suppress or even locally eliminate *Ae. aegypti* populations.

#### **Plant Pests**

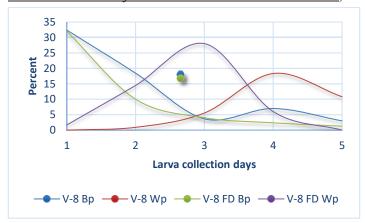
# New improved Mediterranean fruit fly VIENNA-8 genetic sexing strain

In the standard VIENNA-8 genetic sexing strain (GSS) of the Mediterranean fruit fly, males emerge from brown pupae and are resistant to high temperatures while females emerge from white pupae, are sensitive to high temperatures and can be eliminated at the embryonic stage. Females, however, have a slower development rate as compared with males. Recently it was demonstrated that slower development observed during the larval stage is driven by a gene that is not associated with the temperature sensitive lethal (tsl) phenotype. Using classic genetic approaches, a novel white pupae and temperature sensitivity lethal strain fast development (wp tsl FD) was isolated which showed faster larval development in addition to differences in its temperature sensitivity compared with the standard tsl strain. The introgression of this novel wp tsl FD phenotype into the VIENNA 8 strain, resulted in a new strain (VIENNA-8 FD), where females showed a shorter larval development time, differences in the thermal sensitivity and productivity profiles.

Under standard mass-rearing conditions, the females of the *tsl* strain VIENNA-8 need 8 to 9 days to complete their larva development, which is 2 or 3 days more than the males. Faster development of females could allow the optimization and cost reduction of the mass-rearing process allowing females to finalize their development simultaneously with the males (see figure) which will reduce the quantity of larval diet and rearing space needed for medfly production aimed at suppling material for the breeding colonies.

The strain is ready to be tested in mass-rearing facilities and interested Mediterranean fruit fly SIT action programmes should contact Marc Vreysen, Head of the Insect Pest Control Laboratory to request the strain.

More information on the recent publication: (https://academic.oup.com/jee/advance-



article/doi/10.1093/jee/toaa220/5918279?searchresult=1).

Percent larvae collected over time of males (brown pupae, Bp) and females (white pupae, Wp) of the GSS VIENNA-8 (V-8) and the GSS VIENNA-8 FD (V-8 FD) strains.

# The FAO/IAEA/USDA phytosanitary treatment project

Efficacy tests evaluating the relative tolerance to cold treatment of Zeugodacus tau third instars were compared in wild strains of Palampur (India), Fujian (China), and Baipayl (Bangladesh) and a laboratory strain from Fujian (China). Our results suggest that the wild strain from Fujian was more cold-tolerant than Baipayl, Fujian laboratory, and Palampur strains when exposed to < 1.7 °C for up to 15 days. There were no significant differences among the four populations for exposure times of 16 to 20 days. Only one third instar larva from Fujian wild strain survived after 20 days at < 1.7 °C. Confirmatory tests with a large number of Z. tau third instars (> 30,000) from the Fujian wild strain are being conducted to evaluate whether the cold treatment of < 1.7 °C for 22 days is enough to ensure quarantine security for Z. tau. The data generated by these confirmatory tests may support a proposal for a phytosanitary cold treatment targeting Z. tau.



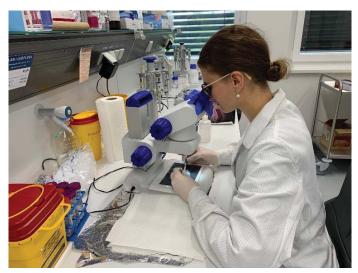
Inajara Viana Gomes Lima and Fabio Luís Galvão, interns, dissecting fruit infested by Zeugodacus tau after exposure to cold phytosanitary treatment.

A manuscript evaluating the effect of normoxia, hypoxia, and severe-hypoxia on the efficacy of phytosanitary irradiation against *Anastrepha fraterculus*, *Anastrepha ludens*, *Bactrocera dorsalis*, and *Ceratitis capitata* was recently published in the journal 'Insects'. This publication supports the International Plant Protection Convention (IPPC) Technical Panel on Phytosanitary Treatments (TPPT) recommendation to remove the restrictions from phytosanitary irradiation treatments under modified atmospheres for tephritid fruit flies. With the rescheduling of the fifteenth session of the Commission on Phytosanitary Measures (CPM- 15), the TPPT recommendation based on our data will be tentatively evaluated by the next CPM in March 2021. The IPCL is currently evaluating the effect of radiation source and dose rate on the survival of *C. capitata* third instars treated with several radiation doses, including doses approved as phytosanitary treatments.

### **Livestock Pests**

# The impact of Iflavirus and Negevirus infection on tsetse mass-rearing

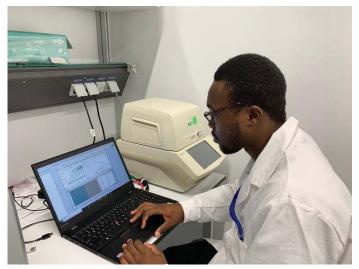
Tsetse flies are harbouring four symbiont bacteria and three groups of viruses that may play an important role in tsetse biology including productivity and performance. The symbionts bacteria are Sodalis, Wigglesworthia, Spiroplasma and Wolbachia. The viruses detected so far in tsetse species are the salivary gland hypertrophy virus (SGHV), Iflavirus and Negevirus. The SGHV negatively affects productivity of tsetse species such as Glossina pallidipes which can destabilise their colonies. However, there is so far no clear evidence of the effect of Iflavirus and Negevirus infection on the performance of tsetse colonies. One of the main reasons to study such viruses is to assess their impact (negative or positive) on tsetse productivity with the aim to improve the performance of the colonies. The screening of the Iflavirus and Negevirus infection in different tsetse species indicated that all tsetse species examined were infected with these two viruses except G. pallidipes, which could indicate that these viruses might protect other tsetse species from SGHV infection. Ms Hannah Huditz, a PhD student from Austria (supervised by Prof. Monique van Oers, Wageningen University and Prof. Wolfgang Miller, Medical University of Vienna) is investigating their mode of transmission and their tissue tropism in the host. Preliminary results indicate that both Iflavirus and Negevirus can be transmitted in tsetse colonies vertically from mother to offspring and horizontally through membrane feeding. Also, the virus infects most of the tissue of the insect host.



Ms Hannah Huditz dissecting tsetse flies to collect different tissues for the detection of Iflavirus and Negevirus.

#### The impact of *Spiroplasma* infection on the performance of *Glossina fuscipes fuscipes* colonies

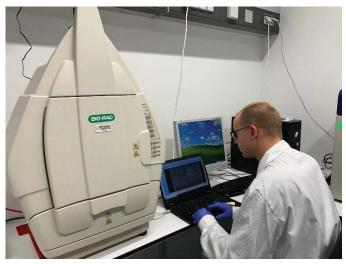
Unlike other tsetse species, adult Glossina fuscipes fuscipes flies from the colony maintained at the IPCL are infected with Spiroplasma. Mr Kiswenda-Sida Mikhailou Dera, a fellow from the Insectary of Bobo Dioulasso (IBD), Bobo Dioulasso, Burkina Faso analysed the prevalence of Spiroplasma infection and its impact on the flies' performance. This work was done in collaboration with Prof. Serap Aksoy and Brian Weiss from Yale University, USA. Preliminary results indicate that Spiroplasma-infected females produce fewer pupae in comparison with uninfected females. The results also indicate that each ovulation cycle in Spiroplasma-infected females was longer than those of uninfected females. Moreover, unmated adults showed a higher density of Spiroplasma than mated adults which might indicate that Spiroplasma reduced the mating ability of the adults.



Mr Kiswenda-Sida Mikhailou Dera analyzing the Spiroplasma density in Glossina fuscipes fuscipes flies using qPCR.

# Development of microsatellite markers for *Glossina austeni* and *Glossina brevipalpis* population genetics study.

The SIT is species-specific and therefore, the mass-reared and released sterile males should be compatible with respect to mating with the target species in the field. Knowledge about the isolation of the targeted populations and potential gene flow with neighbouring populations is important to develop appropriate intervention strategies. Therefore, population genetic studies of the targeted populations are of paramount interest for tsetse SIT programmes. To this end, developing microsatellite markers that can explore the genetic diversity among different population is required.



Mr Fabian Gstöttenmayer screening potential microsatellites using 4% gel electrophoresis.

There are no microsatellite markers available for the two species that are present in South Africa, i.e. *Glossina brevipalpis* or *Glossina austeni*. The development of microsatellite markers for these species has started at the IPCL and is supported by Mr Fabian Gstöttenmayer, an intern from Austria. The preliminary results indicate the availability of more than 10 primers that can be used as microsatellite markers for *G. brevipalpis* and about 7 primers for *G. austeni*. Testing these primers for its suitability for the population genetics study for both species is ongoing.

#### **Human Disease Vectors**

#### Automatic mosquito pupae sex separation impact on purity and male flight ability

The sterile insect technique for disease-transmitting mosquitoes is based on the release of sterile male insects, and this requires the separation of the sexes before release. However, separating millions of male mosquitoes per day for large-scale release operations is challenging. An automatic mosquito pupae sex sorter has been developed by Wolbaki Company (China) and its efficiency was assessed at the IPCL for both Aedes albopictus and Ae. aegypti. The results were encouraging, with less than 1% female contamination in male pupae in both species together with a significant reduction of manpower and increase in time efficiency. However, larvae were observed in male pupae (particularly when the separation is performed less than 24h after first pupation), which could emerge later and increase the female contamination rate. There is thus a need to remove these larvae in the sorted male pupae before male releases. We thus designed an experiment to evaluate the impact of (1) the automatic sex sorting procedure against manual sorting using the Fay-Morlan glass separator on male flight ability; (2) the irradiation of larvae on their viability and the subsequent female contamination rate, (3) two successive sortings on female contamination and male flight ability.

About 80% of the larvae found in male pupae pupated and emerged as adults when cups were left in the emergence cages for 4 days. Of those, about 60% were females and 20% males. However, when *Ae. aegypti* pupae were irradiated with 70 Gy, only 68% of the contaminated larvae reached pupation but failed to emerge into adults and therefore did not increase the female contamination rate. On the other hand, sorting male pupae a second time could effectively remove the contaminating larvae. The automatic sorting did not negatively impact male flight ability in comparison to manual sorting.

The automated sorter is a promising instrument that can separate male and female *Ae. aegypti* and *Ae. albopictus* pupae more efficiently than the manual sorter. However, the operator skills to calibrate the sorter and larvae found in male pupae could be possible limiting factors that must be addressed. Double pupae sorting, irradiation, the identification of the best tilting/sorting time and the removal of the emergence cups before the larvae transform into adults are possible actions that can be undertaken to overcome such limitations.

#### Effects of anoxia on irradiation of adult *Aedes al*bopictus

The effects of anoxia on the dose-response of adult *Ae. al-bopictus* was assessed in a series of small-scale studies in which adult males were irradiated in a Gammacell220 at a diagnostic (non fully sterilizing) dose of 40 Gy whilst anaesthetized in nitrogen in a sealed container (i.e. in anoxic conditions). Longevity was not affected by the anoxia nor the irradiation for the first three weeks at this substerilizing dose, whereas following a high dose (90 Gy), males irradiated in nitrogen showed a significantly higher survival rate. Flight ability, 2 days post irradiation, was not impaired in all treatment groups as compared with the controls.

Although it is still early in the series of experiments, initial results have indicated the possibility that irradiation of adults in anoxia could have a qualitative advantage in *Ae. albopictus*, and possibly other species.

# Sterility of *Aedes aegypti* pupae and adults after irradiation in an X-ray blood irradiator

Both pupae and adults of *Ae. aegypti* were irradiated at increasing doses in a blood X-ray irradiator (Best Theratronics Raycell MK2) to assess the application potential of this irradiation device in SIT programmes against mosquitoes. First results show that the expected induced sterility levels were achieved in both life stages as compared to previous dose-response studies using the RadSource 2400 X-ray irradiator. Adults seem to be slightly more radiosensitive than pupae, and 99.9% sterility was reached with doses of 55 Gy and higher. These data corroborate earlier comparisons of *Ae. aegypti* adult and pupae dose response following irradiation using a Gammacell220.

### Testing a new aerial release device for sterile male mosquitoes in laboratory conditions

The SIT is an effective control method to manage insect populations. Releasing sterile male mosquitoes by air ensures homogeneous coverage, especially over larger areas. We have recently reported on the successful test of a drone in Brazil to release sterile mosquitoes. However, given the restrictive regulation in urban areas with respect to size and weight of drones, a smaller version of a release device for chilled sterile Aedes male mosquitoes was designed (weight <250 g including 50°000 mosquitoes). The device is mainly composed of an insulated chamber that can hold a layer of mosquito less than 45 mm to avoid mechanical damage by compaction (see figure). The device has a release mechanism that accepts pre-set release plans based on a list of geo-referenced waypoints and has its own release rate control. The device was loaded with batches of chilled/marked/compacted/irradiated/ male mosquitoes and their quality was tested under two temperatures (5°C and 26°C) in terms of survival, flight ability and mechanical damage according to the level of compaction (top, bottom) within the release device. The quality was significantly reduced by room temperature whereas no difference was observed between males at different positions (top, bottom) within the holding chamber and the control (non-released males). The chilled release machine will be tested in the field to assess whether it can be used both for ground and aerial releases of chilled mosquitoes.



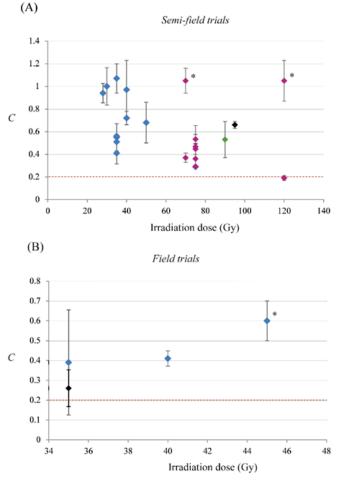
Prototype of new chilled adult mosquito release device for ground and aerial release of sterile males.

#### Competitiveness of irradiated male mosquitoes

Adequate sexual competitiveness of sterile males is a prerequisite to apply the SIT. We reviewed the semi-field and open field trials conducted over the last decade, which demonstrated that irradiated male mosquitoes can be competitive if produced in appropriate conditions.

The competitiveness of a sterile male is the odds of a wild female being mated with a sterile male compared to a wild male when exposed to both in equal numbers and is generally measured through the Fried index (C).

A reduction in quality of the produced sterile male insects may be observed but is generally related to the massrearing, handling, marking and release processes, rather than radiation *per se*. When all these processes are mastered, a good competitiveness of sterile male mosquitoes is observed, and the C value was above 0.2 in all reported studies, which is considered the lower threshold for costeffective projects.



Aedes aegypti Aedes albopictus Anopheles arabiensis Anopheles coluzzii

Competitiveness (C Fried index) of irradiated male mosquitoes measured in semi-field trials (A) and field trials (B), as a function of the irradiation dose (from Bouyer and Vreysen, 2020).

# Reports

### Phytosanitary Treatments Using Irradiation for Fruit Fly Pests Gain Ground

Before fresh fruits and vegetables trek across oceans and borders along international trade routes and to the aisles of neighbourhood grocers, they may be treated to eliminate, inactivate or sterilize pests. This highly regulated practice of disinfestation typically involves phytosanitary, or postharvest treatments, to stop potentially pesky hitchhikers – and is increasingly done using irradiation rather than chemicals.



Female Mexican fruit flies (Anastrepha ludens) prepare to lay eggs into a mango. (Photo: V. Dias/FAO/IAEA).

#### What are phytosanitary treatments?

Phytosanitary treatments are processes that mitigate biosecurity risks – pests and other non-native organisms – from being introduced and established in pest-free areas. "Heat treatment, irradiation and fumigation are conducted before shipment or upon arrival and are usually done in a matter of hours or less," said Scott Myers, Assistant Director of Otis Laboratory at the US Department of Agriculture (USDA). "Cold treatments are typically applied in transit, as they take 12 to 22 days to provide efficacy."

With increasing restrictions placed on the use of chemical fumigants by importing countries, the use of irradiation as a phytosanitary treatment is increasing around the world. It uses ionizing radiation to render pests infertile and "has the benefit of a short treatment time and can maintain the quality of perishable or delicate commodities, such as berries, guavas and figs," Myers said.

Phytosanitary treatment implementation relies on sciencebased agreements between trade partners, a process that can take years to establish. The IAEA, in cooperation with the Food and Agriculture Organization of the United Nations (FAO), established a partnership with the USDA in 2010 to conduct research to help trade and health officials establish standards, as well as understand the role of irradiation as a harmless and environmentally friendly option. "The evaluation of phytosanitary treatment efficacy and the proposal of new treatment schedules require access to a large number of colonized insect pest species and specimens, proper equipment and qualified human resources to be conducted," said Vanessa Dias, an entomologist at the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. "We do this and make our results available for experts around the world."

#### Advancing phytosanitary treatments

"We maintain colonies of many tropical tephritid fruit fly species and populations from around the world, allowing for unparalleled treatment research against plant quarantine pests," Dias added. After ten years and an investment of about US \$2.7 million from the USDA toward research conducted at the IAEA and FAO, resulting scientific data have led to the approval of eight phytosanitary treatments and an additional seven amendments pending approval from the International Plant Protection Convention.

"This work has supported trade agreements through the production of international standards, and irradiation is increasingly recognized as an enabler for meeting phytosanitary safety requirements," Dias said. More fresh fruits from Mexico, for example, can be exported to the United States without the risk of spreading invasive pests as a result of the use of this technology. The USDA estimated that exports of fresh guava from Mexico increased by 52 per cent from 2015 to 2017 thanks to the use of phytosanitary irradiation – the only available treatment for this commodity.

Furthermore, in one study, scientists considered uncertainties related to the efficacy of phytosanitary irradiation doses for insects infesting products stored under low oxygen levels, which limited the application of irradiation. "Our results showed that low oxygen treatments applied before and during irradiation did not reduce the efficacy of irradiation protocols for fruit fly pests," Dias said. "The removal of the restriction to phytosanitary irradiation treatments can increase their applicability and advance the use of nuclear technology for agricultural purposes."

Through the ongoing partnership with the USDA, research will continue to evaluate the effect of physical and biological factors on the efficacy of phytosanitary treatments and to support the development of future international standards for exporting and importing countries. "This cooperation offers a unique opportunity to develop treatments for a wide range of species and populations. We have been able to not only support USDA treatment schedules but also to facilitate adoption of treatments for inclusion in international standards," Myers said.

Source: https://www.iaea.org/newscenter/news/phytosanitary-treatmentsusing-irradiation-for-fruit-fly-pests-gain-ground.

### The Fourth International Meeting of Tephritid Workers of Europe, Africa and the Middle East (TEAM)

The Fourth International TEAM Meeting was successfully held in La Grande Motte, France from 5 to 9 October 2020. The meeting was organized by the researchers of French and Belgium institutions including the Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES), the Centre de Coopération Internationale en Recherche Agronomique pour le Développement National (CIRAD). the Institute De Recherche Agronomique (INRA), the French Ministry of Agriculture and the Royal Museum for Central Africa (Belgium), and chaired by Mrs Valérie Balmès (ANSES). Taking into consideration the travel restrictions due to the worldwide COVID-19 pandemic, the local organizing committee together with the TEAM steering committee decided to organize this event as a combined physical and virtual gathering. Through the sponsorship obtained by the local organizers, the virtual participation was offered for free. As a result, 511 persons from more than 70 countries registered for the webinar, including several delegates from other regional fruit fly networks such as Tephritid Workers of the Western Hemisphere (TWWH) and Tephritid Workers of Asia, Australia and Oceania (TAAO). Although not all registered persons connected, at least 365 delegates actually followed partially or entirely the meeting throughout the week.



The programme of the symposium consisted of plenary speakers and talks, grouped in nine different sessions, over a period of five days, covering all major research aspects. In total, 36 presentations (including two plenary talks) were given throughout the week. Despite the novel approach and the technical difficulties in getting connected with some

participants, all talks could be presented (either live or prerecorded). Posters were submitted in advance and a link distributed to all registered participants prior to the meeting. Forty-four posters were on display and the authors could shortly summarize their findings and reply to questions by the delegates during two poster sessions. This meeting also, for the first time, included explicitly contributions of researchers focusing on the wing-spotted fruit fly *Drosophila suzukii* (Drosophilidae), rather than exclusively topics on tephritid fruit flies. Furthermore, a round table discussion was organized on the theme 'New concepts and approaches in fruit fly management in Europe' which was preceded by short introductory speeches.



Despite the difficult circumstances, this fourth TEAM meeting was considered a great success both by the organizers and the participants. Although physical meetings do have a number of advantages and facilitate interactions and networking, having the option of a virtual attendance and presentation increases the number of people who can partake in the meeting. Members of the local organizing committee and the TEAM steering committee will edit the proceedings of the symposium, which will be published in a separate open access e-issue of the peer review journal Fruits. All relevant information will be made available Symposium's website: through the https://www.alphavisa.com/team/2020/.

#### American Congress on Fruit Flies and 10<sup>th</sup> Meeting of Tephritid Workers of the Western Hemisphere (TWWH)

Under the slogan 'Sharing global solutions for fruit fly management', nearly 1 800 participants from 45 countries and international/regional organizations, virtually attended the American Congress on Fruit Flies and the 10<sup>th</sup> Meeting of Tephritid Workers of the Western Hemisphere (TWWH), from 2–6 November 2020, to promote the exchange of knowledge, state of art technologies and experiences in the management of fruit fly pests among members of the scientific, academic and fruit growers community.

It was a favourable scenario for virtual keynote speeches, oral and poster presentations, discussion panels with fruit fly specialists, fruit growers and exporters as well as exhibitions from suppliers of services and technologies for fruit fly management.



*Virtual platform – American Congress on Fruit Flies and 10<sup>th</sup> Meeting of Tephritid Workers of the Western Hemisphere.* 

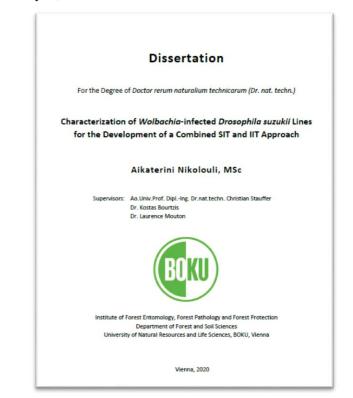
The agenda covered a number of topics related to basic and applied technology including biology, ecology, behaviour, genetics, taxonomy, morphology, attractants, monitoring systems, control methods (including the sterile insect technique), quarantine, postharvest treatments as well as socio-economic aspects, area-wide management and action programmes.

The event was organized by the Instituto Colombiano Agropecuario (ICA), and the Ministry of Agriculture and Rural Development of Colombia and the Tephritid Workers of the Western Hemisphere (TWWH), with support from Asociación Hortofrutícola de Colombia (Asohofrucol), Fondo Nacional de Fomento Hortofrutícola (FNFH), Sociedad Colombiana de Entomología (SOCOLEN), the International Plant Protection Convention (IPPC), the Interamerican Institute of Cooperation in Agriculture (IICA), the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, and companies of fruit fly services and technologies.

"With the realization of this Congress, the objective of the organizing committee was fulfilled which was to have a high participation of professionals, technicians, researchers, as well as producers and exporters looking to create awareness about the importance of fruit flies and the sustainability of action programmes" Emilio Arévalo, the Technical Director for Epidemiology and Phytosanitary Surveillance of ICA and local organizer pointed out that 660 persons from Colombia and some 1100 from abroad attended the event.

### Ms Katerina Nikolouli Obtains her PhD from the University of Natural Resources and Life Sciences, Vienna, Austria

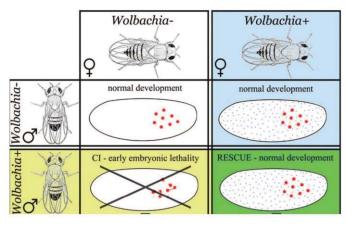
Ms Katerina Nikolouli from Greece successfully defended her PhD dissertation on 9 October 2020 at the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria. Ms Nikolouli did her PhD academic studies at the BOKU and carried out her research at the Insect Pest Control Laboratory (IPCL) in Seibersdorf, Austria and at the Biometry and Evolutionary Biology laboratory, University of Lyon, France.



The PhD thesis entitled 'Characterization of *Wolbachia*infected *Drosophila suzukii* lines for the development of a combined SIT and IIT approach' was supported by a bilateral international (Austria – France) research project named SUZUKILL (Managing cold tolerance and quality of massproduced *Drosophila suzukii* flies to facilitate the application of biocontrol through incompatible and sterile insect techniques). The SUZUKILL consortium involved scientists with multi-disciplinary skills and expertise and the project was focused on the development of biocontrol tools for the management of *D. suzukii* populations in confined areas. All partners produced high-end results that have substantially improved our insights into *D. suzukii* biological control using SIT or SIT/IIT and the research results have been published in peer-reviewed journals.

Ms Nikolouli used in her research two *Wolbachia*-infected *D. suzukii* lines (*w*Ha and *w*Tei) that were evaluated for their ability to induce cytoplasmic incompatibility (CI). Both lines were able to induce CI in *D. suzukii* while *w*Ha expressed higher CI levels. The main goal of the study was

to exploit the SIT in combination with *Wolbachia* symbiosis as a population management approach as a potential component of an area-wide integrated pest management programme. Therefore, the two *Wolbachia* lines were used for the development of a combined SIT/IIT protocol. Three low irradiation doses for *D. suzukii* (45-60-90 Gy) were evaluated, and the egg hatching rates were determined. The results indicated that *w*Ha and *w*Tei females as well as *w*Ha males were fully sterile even at 45 Gy. In addition, the adult emergence, longevity and flight ability of *D. suzukii* were assessed at 45 Gy and no major effect caused by irradiation was detected.



Cytoplasmic incompatibility. There are four different mating combinations between infected and uninfected males and females (image citation: Clark et al. 2005).

The combined SIT/IIT approach could gain on the SIT since the lower irradiation dose might not affect the mating competitiveness of the sterile males, as shown for the mosquito vector species *Ae. albopictus*. Therefore, it is critical to address any questions related to the mating competitiveness of the *Wolbachia*-infected *D. suzukii* males under semi-field conditions prior to the deployment of the combined approach to a large-scale operational programme. The PhD study of Ms Nikolouli, collectively, advanced our understanding of the SIT and IIT 'weaponry' and hopefully, it will help to both manage existing incursions of *D. suzukii* and prepare for future threats.

#### Ms Nicole Culbert Obtains Her PhD from the University of Liverpool, United Kingdom

Ms Nicole Culbert from the United Kingdom successfully defended her PhD thesis on 9 June 2020. She was an offsite student from the University of Liverpool under supervision of Dr Alistair Darby and conducted all her research at the Insect Pest Control Laboratory (IPCL) in Seibersdorf, Austria under the co-supervision of Marc Vreysen, Jeremie Gilles and Jérémy Bouyer.

Ms Culbert's thesis, titled 'Improving the handling, transport and release of sterile male mosquitoes as part of an area-wide integrated pest management strategy' focused on determining the optimal conditions for sterile male mosquitoes during the post-pupal irradiation stages of the sterile insect technique (SIT) package. A range of optimal immobilization temperatures were developed for the storage and transport of male *Anopheles arabiensis, Aedes aegypti* and *Ae. albopictus* wherein it was noted that they could be maintained within the range of 7 - 10 °C for up to 24 hours without any significant impact upon subsequent survival. Furthermore, the impact of compaction during storage was investigated and a maximum tolerable threshold of 1.27 g/cm<sup>2</sup> determined.

A guide to visibly mark all of the aforementioned species using fluorescent dust was designed and validated. A dust quantity of 0.5 mg/ 100 adult males was found to provide a uniform, long-lasting mark without impacting longevity. Additional highlights from her research include the development and verification of flight test devices which can measure the flight ability of sterile male *Ae. aegypti, Ae. albopictus* and *An. arabiensis* and give an indicator of male quality.



Male Anopheles arabiensis during a flight ability test.

The impact of varying environmental conditions relating to the time of day (morning versus afternoon) that sterile male releases could occur was investigated for both male *An. arabiensis* and *Ae. aegypti*. An afternoon release was noted to be less detrimental when considering *An. arabiensis* for release, whilst no impact of time of day was observed in *A. aegypti*.

The volume and quality of the research conducted was impressive, as was demonstrated by the publication of seven scientific papers in various peer-reviewed journals. The data obtained from Nicole's research has, and will continue to aid numerous Member States of the FAO and the IAEA in their quest to develop the SIT package and begin the operational phase of releases in the coming years in the fight against human disease vector.

### Announcements

#### Animated Infographic on How to use Fruit Fly Standards to Gain Market Access

An animated infographic developed in collaboration with the International Plant Protection Convention (IPPC) on 'How to use fruit fly standards to gain market access' was published in six languages (English, Arabic, Chinese, French, Russian and Spanish). Animated Infographic can be found at (<u>http://www-naweb.iaea.org/nafa/resourcesnafa/multimedia.html</u>).

The IPPC it's a multi-lateral treaty that facilitates global cooperation to protect plants from harmful pests, such as fruit flies: pests that may be introduced and spread through international trade. Fruit flies present a major challenge to international trade, because if they are detected in fruits, trade may be stopped. That is why a set of International Standards for Phytosanitary Measures (ISPMs) on fruit flies has been developed and recently reorganized. When a country wants to export fruit, this set of standards can facilitate the process.

Those include ISPM 37 on 'Determination of host status of fruit to fruit flies (Tephritidae)'. This standard provides guidelines for the determination of host status of fruit to tephritid fruit flies and describes three categories of host status of fruit to fruit flies (Classification of a plant species or cultivar as being a natural host, conditional host or nonhost for a fruit fly species). It includes methodologies for surveillance under natural conditions and field trials under semi-natural conditions that should be used to determine the host status of undamaged fruit to fruit flies for cases where host status is uncertain. Can a fruit be a host for fruit flies? In another words, if the fruit is not susceptible to the fruit fly, the pest cannot infest it. It is a non-host and therefore it can proceed directly for export.

Next comes ISPM 26 on 'Establishment of pest free areas for fruit flies (Tephritidae)'. This standard provides guidance for the establishment of pest free areas (An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained) for tephritid fruit flies of economic importance, and for the maintenance of their pest free status. If a fruit comes from a fruit fly free area, then it can go for export. But if fruit flies are present in the area, then the country or area must apply additional phytosanitary measures, to reduce the risk of fruit fly hosts introducing the pest to the importing country. This scheme is reflected on ISPM 35 'Systems approach for pest risk management of fruit flies (Tephritidae)'. It provides guidance for the development, implementation and verification of integrated measures in a systems approach as an option for pest risk management of tephritid fruit flies of economic importance to facilitate trade of fruit fly host products or to minimize the spread of regulated fruit flies within an area. In a systems approach (A pest risk management option that integrates different measures, at least two of which act independently, with cumulative effect), the integration of these measures will reduce the risk of pests in fruit trade to levels that are acceptable to the importing country, while contributing to assuring high quality of the fruits to be exported.

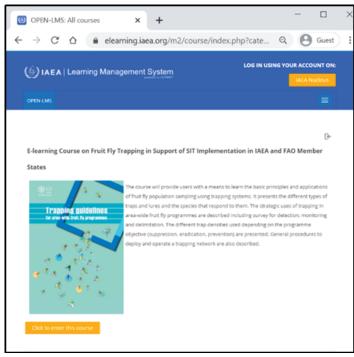


In conclusion, the phytosanitary scheme under which trade might occur is defined and agreed by the interested parties, based on ISPMs. This is done through a bilateral agreement between the plant protection organizations of both the exporting and the importing countries. Through the implementation of the ISPMs, countries protect plant resources from fruit fly pests while, at the same time, they ensure the application of justified measures that facilitate safe trade.

### **E-learning Course on Fruit Fly Trapping in Support of Sterile Insect Technique Implementation**

This e-learning course is based on the 'Trapping Guidelines for Area-Wide Fruit Fly Programmes', Second Edition, published by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture in 2018.

It provides users with a means to learn the basic principles and applications of fruit fly population sampling using trapping systems. It presents the different types of traps and lures and the species that respond to them. The strategic use of trapping in area-wide fruit fly programmes are described including survey for detection, monitoring and delimitation. The different trap densities used depending on the programme objective (suppression, eradication, prevention or containement) are presented. General procedures to deploy and operate a trapping network are also described.



The aim of the e-learning is to understand the principles behind trapping. By successfully completing this e-learning course you will be able to apply the lessons learned including in real life operational programmes.

The course is divided into 5 modules, each containing one or more chapters. Furthermore, a list of appendixes containing additional information as well as all references and glossaries is available throughout the whole course. The course presents videos illustrating how trapping applications are integrated and used in fruit fly action programmes

This e-learning course does not have a time limit. Therefore, you can start the course at any time, take breaks from it and continue where you have left. The course can be accessed through IAEA e-learning platform: <u>https://elearning.iaea.org/m2/course/index.php?categoryid=</u> <u>50</u>.

### Harmonized Identification Guideline of Tephritids that Might be Considered of Economic and Quarantine Importance in Latin America and the Caribbean

The correct identification of endemic fruit fly pests is fundamental for the design of effective integrated pest management programmes including those that apply the sterile insect technique (SIT).



Also, for the enforcement of emergency action plans when incursions of invasive fruit fly species of quarantine importance occur in an area. This manual is aimed at offering National Plant Protection Organizations (NPPOs) in the Latina American and Caribbean countries with a harmonized guide that can support them in the continues battle against fruit fly pests affecting food security and safety in the countries.

This guideline was prepared under the IAEA Technical Cooperation Project RLA5070 jointly with the Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) and it is only available in Spanish. It can be downloaded at IPC website: <u>http://www-naweb.iaea.org/nafa/ipc/public/Guia210220.pdf.</u>

### Guidelines for Irradiation of Mosquito Pupae in Sterile Insect Technique Programmes

The Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture has been investigating the use of nuclear techniques to manage mosquito vectors in a sustainable, environment-friendly manner, by developing the sterile insect technique (SIT) package for species such as *Aedes aegypti, Ae. albopictus* and *Anopheles arabienss*.



The key to this technique is the induction of reproductive sterility in the male mosquitoes which are to be released into the target site where population suppression is intended. Therefore, it is essential to ensure that the methods of the sterilization processes are optimal in inducing the desired effects, whilst minimizing detrimental effects which could decrease the biological quality of the released males.

This publication is intended as guidance for the irradiation of the pupal stage of *Ae. aegypti*, *Ae. albopictus* and *An. arabiensis*, for routine studies on the biological effects of radiation exposures.

The guideline can be downloaded at IPC website: <u>http://www-naweb.iaea.org/nafa/ipc/public/2020-</u> <u>Guidelines-for-Irradiation.pdf</u>.

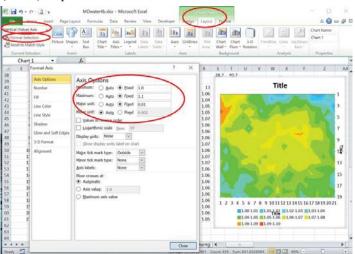
### Frequently Asked Questions (FAQs): Mosquitoes

One essential component for the preparation and enhanced success of sterile insect technique (SIT) pilot trials against mosquitoes and continuous reinforcement of further SIT programmes, is the understanding, acceptance, and support of all stakeholders. It is therefore valuable to channel the key messages to the stakeholders in a scientifically accurate, truthful, and understandable manner. Being prepared to answer frequently asked questions (FAQs) concisely and unambiguously will help convey a feeling of trust and confidence and will greatly benefit the SIT programmes. Here we present some FAQs requested by our counterparts (https://nucleus.iaea.org/sites/naipc/dirsit/Pages/Sterile-

<u>Insect-Technique-for-Mosquitoes.aspx</u>) that are often encountered during community engagement activities with proposed answers, and a guidance to 'Do's and Don'ts' when communicating key messages before and during SIT activities.

#### Dose Mapping by Scanning Gafchromic Film to Measure the Absorbed Dose of Insects During Their Sterilization

Dose mapping provides operators of irradiators used for the sterile insect technique (SIT) with the information of the dose within the irradiation container, including areas of maximum and minimum dose, the dose uniformity ratio (maximum dose/ minimum dose), and areas were the dose rate is relatively uniform so that a suitable volume for the canister can be selected to provide the dose uniformity ratio (DUR) required.



The absorbed dose that is used to induce sterility is of prime importance to programmes that release sterile insects. Absorbed dose can be measured using any dosimetry system, but many dosimeters are relatively large limiting the resolution that can be achieved. Radiochromic films can be used to measure dose over the area of the sheet used and have good resolution, in the order of tens of micrometres.

The development of better system for dose distribution within an irradiation container and the development of an accurate dose-response curve for the target insect using precise dosimetry is a prerequisite of any programmes releasing sterile insects. This manual describes the operational procedures to develop dose maps by scanning Gafchromic film and the calibration of the system, to be used in the insect irradiation process for SIT programmes.

The manual is now available in English and Spanish. Both versions can be downloaded from IPC website at <u>http://www-naweb.iaea.org/nafa/ipc/public/Dose-Mapping-Gafchromic-2020-11-02.pdf</u>.

### In Memoriam

#### Hernán Donoso Riffo (1954-2020)

With great sadness, we announce the passing away of Mr. Hernán Donoso Riffo, a biologist and researcher of the Servicio Agrícola y Ganadero (SAG), Arica, Chile, on 10 September 2020.



Hernán graduated in Biology and spend most of his career working for SAG, the institute of which he belonged for more than 30 years. When joining the SAG in 1988 he worked in the fruit fly control programme on receiving and performing quality control tests of sterile flies shipped from Hawaii. He then joined the Mediterranean fruit fly production and sterilization centre in Arica that was dedicate to the use of the sterile insect technique (SIT), becoming the head of the centre for more than five years.

Since his initial years he participated in entomological projects including research projects such as Coordinated Research Projects (CRPs) on 'Quality Assurance of Mass Produced and Released Fruit Flies', 'Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application', 'Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control', and 'Improved Field Performance of Sterile Male Lepidoptera to Ensure Success in SIT Programmes' of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. Hernán also lead the project 'Screening of Fruit Fly Attractants and its Association with Methodologies for the Control of the Mediterranean fruit fly' from the 'Fund for Improvement of the Sanitary Patrimony', Foundation for Fruticulture and Development and the 'Fundación para la Innovación Agraria' Project on 'Development of the Sterile Insect Technique (SIT) Through Ionizing Radiation for the Control of the Quarantine Pest *Lobesia botrana*'. He also led the implementation of the IAEA Technical Cooperation Project on 'Implementing Pilot Level of Sterile Insect Technique for Control of *Lobesia botrana* in Urban Areas'.



Based on Hernán's ample knowledge and experience, in 2015 the experimental laboratory on *Lobesia botrana* was established. He directed the research achieving the rearing of this insect under laboratory conditions and initiating a pilot test for the control of *L. botrana* in urban areas. This was the first testing of SIT for *L. botrana* species.

Hernán is leaving a deep empty space among all those who collaborated with him. We will honor him by keeping in our memories his happy collaborative spirit and vast knowledge and contributions to the research and application of the SIT.

Source: Melissa Salazar Sepúlveda, Centro de Producción de Insectos Estériles. (CPIE), Servicio Agrícola y Ganadero Región de Arica y Parinacota, Servicio Agrícola y Ganadero (SAG), Arica, Chile. I, Greg Simmons, first met Hernán working with him within the IAEA CRP on 'Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control' which SAG had joined as part of their effort to develop the SIT for use as an Integrated Pest management (IPM) tool to combat L. botrana affecting grape and fruit producers in Chile. In 2015, I was able to attend the opening of the new L. botrana facility in Arica and got a chance to see the activities in the facility and learn about the goals of the programme. On subsequent visits, I saw the rapid progress made under Hernán's leadership to develop the mass-rearing system and the implementation of field pilot evaluations. In just a few years, the project went from developing rearing systems and quality control systems, to completing radiation biology studies, to season-long implementation of field evaluations in grape production areas in the center of the country.



Hernán brought the perspective from a long career working in methods development on Mediterranean fruit fly SIT and plant protection to the work on *L. botrana*. One of Hernán's focuses was to develop a mass-rearing larval diet that could produce large numbers of high-quality moths at a cost that the programme could support. His driving principles was a focus on finding ingredients that were available in Chile at reasonable cost. This is what led him, at my last count, to develop and test 48 separate *L. botrana* diets. When I asked him what was wrong with diet number 33 that I thought had been performing very well, his simple reply was "this one has cheaper ingredients and I can source all of them from Chile".



Hernán had absolute dedication to the support and training of his staff and to the field of SIT and insect mass-rearing. These traits allowed him to bring the SAG team together to develop the programme so quickly. Hernán was a warm person and had a great sense of humor with his jokes about the quirky side of life and the life of an entomologist that kept us amused. Besides the technical nature of our visits, he also made sure as a visitor that we got to experience authentic northern Chilean culture, the interesting life of the people in Arica and the wonderful food. I don't think I ever suffered for lack of a good meal or a glass of Chilean wine in Hernán's company. As his friend and colleague from USDA, I will miss him and I want to extend my deepest sympathy and condolences to his family, friends and colleagues.

Source: Greg Simmons, USDA-APHIS-PPQ, USA.

# **Other News**

#### Nature Plants Publishes A New Paper on Science Diplomacy for Plant Health

Nature Plants published a new paper on Science Diplomacy for Plant Health, co-authored by leading plant health community representatives. International research collaboration is key to fight plant pests and diseases that cause up to 40 percent of food crop losses and USD 220 billion in losses every year, leaving millions of people without enough food to eat and seriously damaging agriculture. The IPPC Strategic Framework 2020–2030 foresees a development agenda on global phytosanitary research coordination, whose work is kicked off with the current study.

The IPPC community is excited to announce the release of a new policy paper on 'Science diplomacy for plant health'. The study, published by the prestigious peer-reviewed scientific journal Nature Plants, was developed at the occasion of the International Year of Plant Health 2020. It brought together qualified experts from the international plant health community to analyse new perspectives and challenges on global phytosanitary research coordination.



"Science is the key to build synergies between national and international communities. Establishing a global research network of phytosanitary experts and researchers, and boosting international collaboration, is crucial to help both local and international authorities fight plant health threats, and find common solutions to emerging global challenges", stated one of the co-authors, Dr. Jingyuan Xia, IPPC Secretary Officer in Charge and Director of FAO Plant Production and Protection Division. The establishment of a global network aiming at facilitating international research efforts on regulated and emerging pests can overcome some of the limitations phytosanitary systems are currently experiencing. It also helps better protect countries and their agriculture, environment, and trade activities from plant pests and diseases. Such a global network should facilitate collaboration between policy makers, research funders and scientists and bring together national and regional research authorities such as National Plant Protection Organizations (NPPOs), Regional Plant Protection Organizations (RPPOs), governments, as well as representatives from academia, research institutes, and industries.

"As the complexity of plant health challenges requires knowledge and specialized expertise that cannot be found in a single country alone, diplomacy for science triggers international multi-disciplinary collaborations to tackle these challenges", said Dr Baldissera Giovani, Euphresco coordinator and lead author of the study. "Global phytosanitary research coordination will speed-up harmonization of approaches and reconcile national views to move from the lowest common denominator compromise to a more ambitious vision of international plant health", he added.



The need for a global research coordination network has been already identified by the phytosanitary authorities in many countries. The IPPC Strategic Framework for 2020-2030, which will be presented for adoption at the fifteenth session of the Commission on Phytosanitary Measures (CPM-15) in 2021, addresses this issue. Much still needs to be done, and the plant health community is thrilled to start looking into new solutions and approaches to strengthen research coordination at the global level.

The article can be viewed from https://rdcu.be/b6crb.

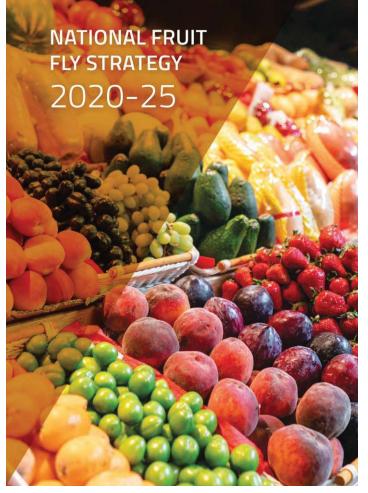
For more information, visit https://www.ippc.int/en/.

Source: IPPC news, 11 August 2020. (https://www.ippc.int/en/news/nature-plants-publishes-a-new-paper-onscience-diplomacy-for-plant-health/).

# Australian National Fruit Fly Strategy 2020-2025 now available

The Australian National Fruit Fly Strategy 2020–2025 (NFFS) has been developed by Australian National Fruit Fly Council (the Council) to provide a framework for ongoing stakeholder cooperation to support a contemporary, viable, cost-effective and coordinated national approach to fruit fly management. The strategy applies to all endemic and non-endemic species of fruit fly.

A draft NFFS was released in 2008, and since that time there have been a number of challenges to Australia's fruit fly system including the loss of key disinfestation chemicals, changes to fruit fly distributions, and increasing scrutiny and assurances being sought from trading partners. There has also been important progress made in strengthening the fruit fly system, such as in sterile insect technique, irradiation treatment, diagnostic tools, area wide integrated pest management, and systems approaches.



The NFFS has been revised and streamlined to incorporate these developments and align with other national strategies such as the National Plant Biosecurity Strategy, National Plant Biosecurity Diagnostic Strategy, and the National Plant Biosecurity Surveillance Strategy.



Queensland and Mediterranean fruit fly distribution in Australia (Image courtesy of Australian Government Department of Agriculture, Water and the Environment).

The strategic objectives of the 2020–2025 NFFS are to:

- maintain Australia's freedom from exotic fruit fly.
- minimise the incidence and spread of fruit fly.

- implement national systems that support market access.
- facilitate a cooperative and committed national approach to fruit fly management.

Eight different, yet interdependent, priority areas are also identified in the strategy: market access; management of established fruit fly; prevention, preparedness and response; research; surveillance; diagnostics; communication and engagement; and cooperation.

The PDF version of the Strategy can be downloaded at: <u>https://preventfruitfly.com.au/wordpress/wp-</u>

<u>content/uploads/2020/11/National-Fruit-Fly-Strategy-2020-25.pdf</u>.

Source: <u>https://preventfruitfly.com.au/national-coordination/national-fruit-fly-strategy/</u>.

#### Aerial Drop of Sterile Queensland Fruit Flies Proves a Winner

# Aerial releases of sterile fruit flies are proving effective in the fight against the fruit pest.

Sterile fruit flies have been released from a plane during a pilot program, every week over an eight-month period in Hillston, New South Wales (NSW) and Cobram, Victoria, Australia.

The Post Factory Pilot of SITPlus Fly production has had a positive impact according to Cobram agronomist, Russell Fox. "The sterile fruit fly releases have worked well so far and are widely supported by local growers and community," Mr Fox said. "The program is a very useful complement to the regional fruit fly management strategy."

Goulburn Murray Valley regional fruit fly coordinator, Ross Abberfield, said the timing of the SITPlus project was ideal, fitting in with the area wide management activity. "In Cobram, the sterile fruit fly releases contributed to an 83 per cent reduction in Queensland fruit fly activity," Mr Abberfield said.

Plant and Food Research New Zealand Plant and Food Scientist, Lloyd Stringer said the rate of capture of wild male Queensland fruit fly in traps was on average 10 times higher in Mooroopna than in Cobram where sterile flies were being used.

In Hillston, initial analysis indicates wild Queensland fruit fly (Q-fly) were detected in very low numbers in town and almost undetected on nearby farms. Residents claimed they were able to eat their backyard fruit for the first time in years.

The Hort Innovation-funded project was a pilot that enables grower groups to confidently consider the sterile insect technique (SIT), for management of Q-fly.



The release of sterile male Queensland fruit fly has proven effective in helping control the pest in the key horticulture areas of Hillston, New South Wales and Cobram, Victoria.

The project was testing efficacy of Q-fly SIT in the field and rearing sterile flies in localised centres. It was also testing the development of quality control procedures in the dedicated rearing out centres.

Hort Innovation SITPlus Program's Director Dan Ryan said "Once released in the environment, the sterile insects' mate with their wild counterparts which disrupts reproduction and suppresses pest population numbers."

Mr Abberfield said in the Goulburn-Murray Valley, an area-wide management program coordinated through Moira Shire Council has been in place since June 2017. "This activity has included the removal of feral fruit trees, abandoned orchards, and neglected urban trees on both public and private land," he said.



Hort Innovation SITPlus Program's director, Dan Ryan, says the sterile insect technique is based on the mass rearing, sterilisation, and release of targeted pest insects.

"This non-SIT area-wide management program activity has shown that Queensland fruit fly activity has reduced by 57%." Mr Ryan said the area-wide program was an important contribution to achieving Cobram's outstanding success. Season two of the SITPlus Pilot began in mid-September and will continue through to April 2021.

Source: North Queensland Register News, 26 October 2020. (https://www.northqueenslandregister.com.au/story/6919179/aerialdrop-of-sterile-fruit-flies-proves-a-winner/?cs=4735).

### 100 Million Sterile Mediterranean Fruit Flies Unleashed on Adelaide Metropolitan Area, Australia

Almost 100 million super-attractive sterile fruit flies will be released across Adelaide's suburbs over the coming weeks in an all-out assault against eight current metropolitan fruit fly outbreaks. The sterile insect technique (SIT) flies will be unleashed on outbreaks in Blair Athol, Croydon Park, Angle Park, Rosewater, Semaphore Park, Pooraka, Campbelltown and Klemzig - impacting more than 250 suburbs across Adelaide.

Minister for Primary Industries and Regional Development David Basham said the use of SIT flies is a key weapon in helping to eradicate the metropolitan outbreaks. "With eight outbreaks across metropolitan Adelaide we must use every tool at our disposal to defend against this devastating plant pest," said Minister Basham. "The use of SIT flies has played an important role in eradicating previous fruit fly outbreaks in South Australia. It is expected the release of 100 million sterile flies will help to knock out the Mediterranean fruit fly population in Adelaide as we come into the warmer, more active months for fruit flies. "The Marshall Liberal Government is committed to eradicating fruit fly to protect the state's \$1.3 billion fruit fly vulnerable horticulture industry.

"The clean-up of ripe fruit across the suburbs and organic pesticide has reduced the wild fly population and now the SIT is being brought in to wipe out any remaining wild flies in the outbreak areas. Once released, the SIT flies mate with any remaining wild flies, with millions of sterile flies they simply overwhelm the wild fly population. "We have eight outbreaks across metropolitan Adelaide impacting suburbs from the beach to the hills and from Cross Road to the northern suburbs and we need to do everything we can to get rid of this pest.

"Our fruit fly pest free status gives our food producers a market advantage around the world and enables them to grow fruit without the need for pesticides or costly treatments. Not only can fruit fly be devastating for our primary producers but as we are seeing right now it has a huge impact on everyday backyard growers. "With the long weekend coming up it's crucial everyone plays their part in keeping South Australia fruit fly free. I'd encourage everyone to remove all ripe fruit from trees, pick up any that has fallen to the ground and do not travel across the state with any fresh fruit or vegetables. We appreciate the ongoing co-operation of residents in the impacted areas to get on top of these fruit fly outbreaks."

Quarantine restrictions in affected areas are due to be lifted without further detections by December 2020.

Source: FreshPlaza News, 2 October 2020. (https://www.freshplaza.com/article/9255464/au-100-million-sterilefruit-flies-unleashed-on-metro-outbreaks/).

#### Pest Alert: Cactus Moth Spotted in Texas

The cactus moth (*Cactoblastis cactorum*, Berg) has been found in the Upper Coastal Texas Counties of Brazoria, Chambers, Colorado and Matagorda. Said by Commissioner Sid Miller, Texas Department of Agriculture (TDA) and Sheri Bethard, Texas Certified Master Gardener "If you spot this pest, immediately report to the TDA Plant Quality Program by calling 512 463-7660 or email PlantQuality@TexasAgriculture.gov. I have seen numerous stands of prickly pear cactus around our county. I know of some on Ferry Drive in Bridge City and some on Higway 105 S. in Vidor. I am sure there are more around the county that I have not seen so we all need to be vigilant in watching for this pest."

The cactus moth is a major pest of prickly pear cactus (*Opuntia* spp.). Cactus moth larvae live and feed inside the pads of prickly pear cacti. Voracious feeding by cactus moth larvae results in the destruction of entire stands of prickly pear cactus.



Adult cactus moths, female on left, male on right.

Native to South America, the cactus moth first appeared in the USA in 1989 in the Florida Keys. Since its initial introduction, the cactus moth has spread throughout the coastal areas of the southeastern USA. Cactus moth was first detected in Texas in 2017 in Brazoria county and has since been found in Matagorda, Colorado, and Chambers counties. Adult cactus moths have been known to disperse naturally a maximum of 16 miles. Commercial sales of infected cacti, as well as weather events, could spread the larvae and eggs greater distances.

This invasive pest gravely threatens the native prickly pear cacti in Texas. The TDA urges nurseries, county extension agents, master gardener associations, and the general public to actively scout for this pest on prickly pear cactus. Be aware there are a number of native species of Lepidoptera larvae that can be found feeding on prickly pear and may be confused with *C. cactorum*, so correct identification by a qualified entomologist is important.

To prevent further spread of cactus moth in the USA, it is very important to detect, report and rapidly respond to all new outbreaks of the pest. The first priority must be to make a serious attempt to eradicate such infestations. Until sterile insect release technology and other control methods are developed and tested by the USDA Agriculture Research Service and other groups, confirmed infestations should be eradicated by manual removal and destruction of egg-sticks and infected cacti stems. Effective control of the cactus moth using insecticides is still in the testing phase.

#### How to Spot the Cactus Moth

How to spot this invasive pest?

1. Check for larvae.

The larval stage is the most distinctive life stage of the cactus moth. Mature larvae are reddish-orange with black spots forming transverse bands.



*The larvae of cactus moth.* 2.Check for damage to cactus pads.

Cactus moth larvae internally feed on the pads of prickly pear cacti. Damaged pads will show

characteristic oozing of plant juices and insect excrement.



Prickly-pear cactus pad with early evidence of a cactus moth infestation. A coin-sized tan patch with 5-9 exit holes surrounds the original entry. These small holes are used by larvae to expel droppings.



Damaged prickly-pear cactus pad caused by internal feeding of cactus moth larvae.

#### 3. Check for egg sticks.

Adult cactus moths lay chains of eggs called egg sticks that resemble the naturally occurring spines on prickly pear pads. The egg sticks are distinguished from spines by their curved appearance.



Cactus moth egg stick on spine of prickly pear cactus.

Source: The Orange leader News, Dawn Burleigh, 13 June 2020. (<u>https://www.orangeleader.com/2020/06/13/pest-alert-cactus-moth-spotted-in-texas/</u>).

### **Relevant Published Articles**

#### X-Ray-Based Irradiation of Larvae and Pupae of the Navel Orangeworm (Lepidoptera: Pyralidae)

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#### Abstract

The suitability of adult male the navel orangeworm, Amyelois transitella (Walker) for Sterile Insect Technique (SIT) has been reported for both high energy gamma (>1 MeV) and low energy x-ray (90 keV) sterilization. However, research regarding sterilization of NOW larvae and pupae by gamma irradiation indicated nonsuitability due to high mortality. Here, NOW larvae and pupae were irradiated to doses up to 50 Gy with 90 keV x-rays, then paired with nonirradiated colony mates. Sterility of surviving insects was determined by the presence or absence of hatched neonates. While presence of offspring does not guarantee viability, the absence does guarantee sterility (as is appropriate for SIT) and was thus the measure used here. Early stage larvae experienced 77% mortality at a dose of 30 Gy, versus 20% for nonirradiated control. At 40 Gy, mortality reached 98%. Of surviving early stage larvae at 30 Gy, 29% of moth pairs produced offspring. For late stage larvae, no offspring were produced at 40 Gy, but mortality was 73%. For pupae, mortality reached 53% at 30 Gy with 13% still producing neonates, while mortality reached 98% at 40 Gy. These results are consistent with reported results for gamma irradiation of NOW larvae where sterility was observed somewhere between the 30 Gy and 60 Gy data points, but mortality was high. This further confirms the lack of suitability of NOW irradiated in the larval stage, whether by gamma or x-ray, and supports the hypothesis that x-ray and gamma treatments are biologically equivalent at equal doses.

The full paper was published in: Journal of Economic Entomology, Volume 113, Issue 4, August 2020, Pages 1685–1693, <u>https://doi.org/10.1093/jee/toaa111</u>.

#### **Sweeping the Flies Away: Evidence From a Fruit Fly Eradication Program**

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#### Abstract

This article evaluates the short-term impacts of a fruit fly integrated pest management program in Peru. Exploiting arbitrary variation in the program's intervention borders, we use a geographical regression discontinuity design to identify the program's effects on agricultural outcomes. Pre-treatment balance tests show that producer and farmlevel pre-treatment characteristics evolve smoothly at the intervention border. Results indicate that farmers within treated areas improved pest knowledge and are more likely to implement prevention and control practices. Also, they increased fruit production and sales. Our findings are confirmed by placebo tests and are robust to alternative regression discontinuity bandwidths and polynomials.

The full paper was published in: European Review of Agricultural Economics, Volume 47, Issue 5, December 2020, Pages 1920–1962, <u>https://doi.org/10.1093/erae/jbaa015</u>.

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