



Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Insect Pest Control Newsletter



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To Our Readers



Dear readers, the newsletter of the Insect Pest Control (IPC) Subprogramme of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture has reached the 100th issue. For this special occasion we have prepared an additional section with articles published in the period from 1997 to 2022. The compilation consists of selected articles with high impact field projects that integrate the sterile insect technique (SIT), relevant research and development as well as major publications.

The cases were selected based on the impact that the projects have had on food security and livelihoods of people in FAO and IAEA Member Countries. Some of the projects and research were initiated before 1997 but have remained active producing relevant results which have continued to be reported in the period covered.

Another important aspect of the field projects, research and development are the long periods until the results are achieved. As an example, China recently opened the market for cherries from Argentina, which is a result of the work initiated in 1992 through an IAEA Technical Cooperation Project with the objective of suppressing the populations of Mediterranean fruit fly (*Ceratitis capitata*) in Patagonia. This evolved later for the establishment of fruit fly free areas in Argentina that are now recognized and key for the market access. Also, most of the readers are aware that the Mediterranean fruit fly genetic sexing strain with the temperature sensitive lethal (*tsl*) mutation (VIENNA 8) took more than 25 years to be developed, but right now it is in use in almost all the Mediterranean fruit fly facilities around the world. More than 4 billion sterile flies are produced weekly by using this strain representing an enormous economic benefit for the mass-rearing of those insects.

Additionally, I would like to highlight a recent success on the eradication of an extensive outbreak of Mediterranean Fruit Fly from the State of Colima in Mexico that was officially announced on 2 August 2022 by the Secretary of Agriculture of Mexico.

The Colima outbreak, detected in April 2021 in the country's largest port, Manzanillo, posed an immediate risk to crops such as carambolas, figs, guavas, mangoes, papaya, pink grapefruits and oranges. If not managed promptly, Mexico, the world's seventh-largest producer and exporter of fresh fruits and vegetables, could face quarantine restrictions imposed by countries free from this pest. This could affect the country's trade in such goods, which generates over 189 billion Mexican pesos (8.8 billion Euro) annually in export, as well as millions of local and rural jobs.

A swift and effective emergency response, building on years of the Joint FAO/IAEA Centre assistance, is helping the Mexican state of Colima to avoid significant economic damage from an outbreak of the Mediterranean fruit fly.

Responding to a request for support from the Mexican government, the Joint FAO/IAEA Centre, over a period of 13 months after the outbreak detection, has assisted the national plant protection authorities in Colima in bringing the outbreak towards an end. The assistance has included developing and providing guidance for the execution of an emergency action plan using a nuclear technique which serves as a birth control for pests.



A release box containing some 15 million sterile male Mediterranean fruit flies being loaded to a Cessna aircraft for release over Colima, Mexico. (Photo: DGSV Senasica).

The detection of this pest in Colima in April last year, 1 300 kilometres from the closest wild populations located in the state of Chiapas, at the border area between Mexico and Guatemala, was alarming, but it was controlled and further eradicated.

The implementation of an emergency action plan included the SIT as the main tool used to eradicate the outbreak. This insect population eradication relied on the release of sterilized male flies. The SIT is among the most environment-friendly insect pest control methods available, and the Joint FAO/IAEA Centre supports countries, including Mexico, in reducing and eradicating the Mediterranean fruit fly population.

Please see the full story in this newsletter as one of the impacts achieved in the field.

Rui Cardoso Pereira
Head, Insect Pest Control Section

Main Achievements of the Past 25 Years

I. Field Projects

Moscamed Regional Programme (1977–2022)

The Mediterranean fruit fly, *Ceratitis capitata* (Wied.) reached Guatemala and Mexico in 1976 and 1977, respectively. In response, the governments of Guatemala, Mexico, and the USA joined efforts to (1) contain further northward spread of the pest, (2) eradicate it from the areas it had invaded in southern Mexico, and (3) in the longer term eradicate it from Guatemala and eventually from the rest of Central America. The programme was the first area-wide large-scale application of the sterile insect technique (SIT) against this pest.



The state-of-the-art Mediterranean fruit fly mass-rearing and irradiation facility with the capacity of producing one billion sterile males per week, inaugurated on 29 August 2021 in Metapa de Dominguez, Chiapas, Mexico (Photo: SENASICA).

The SIT was transferred to Mexico as early as 1978 through an IAEA and FAO technical cooperation project. This allowed the construction and operation of the largest fruit fly mass-rearing facility of that time with a production capacity of 500 million sterile flies per week located in the town of Metapa de Dominguez, Chiapas, Mexico, and few years later the El Pino facility in Guatemala that became the new largest facility with a production capacity of 2 billion sterile flies per week. Both facilities have been the cornerstone of the Moscamed Programme supplying sterile flies for over 40 years, first, to eradicate the pest from the southern state of Chiapas in 1982 and then to maintain a sterile fly biological barrier between Guatemala and Mexico. In August 2021, the old Metapa facility was replaced by a state-of-the-art facility with a production capacity of 1 billion sterile flies per week. For four decades, the programme has served as an effective containment barrier protecting the assets of horticultural producers and contributed during this period to the development of multibillion dollar export industries in these countries.

Chile National Fruit Fly Programme (1995–2022)

Chile's fruit fly-free status has allowed one of the most important export-oriented horticulture industries in the world to develop. To protect this valuable asset, in 1980 the Government of Chile, through the Servicio Agrícola Ganadero de Chile (SAG) of the Ministry of Agriculture (MAG), created Chile's National Fruit Fly Programme. In the mid 1990's, SAG decided to introduce the sterile insect technique (SIT) as a strategic tool against invasive fruit fly pests including the Mediterranean fruit fly.



Sterile Mediterranean fruit fly production facility in Arica Chile (Photo: SAG Chile).

In 2019, twenty-six years after Chile declared itself medfly free, the country experienced one of its largest outbreaks. Worried about future outbreaks, which are caused by the movement of people and infested fruit from countries where the with medfly is present, in 2020 Chilean experts from the Chile's SAG, in collaboration with Argentina's National Food Safety and Quality Service (SENASA), the IAEA and the FAO, decided to apply the SIT as a preventive measure. The sterile flies are shipped from the facility located in Arica north of the country to be released over the areas at risk in the Metropolitan Region of Santiago. The release of sterile flies produced locally is complemented with sterile flies imported from a medfly facility in Argentina. Chile's National Fruit Fly Programme has been the driving force behind the expansion of the fruit and vegetable export industry, one of the main contributors to the country's gross domestic product (GDP).

Argentina's National Fruit Fly Control and Eradication Programme (PROCEM) (1998–2022)

The Mediterranean fruit fly, *Ceratitis capitata* (Wied.) arrival in Argentina affected both fruit production and trade. The pest was initially controlled using conventional insecticide sprays in fruit orchards and a postharvest treatment using a fumigant. However, in addition to these treatments costing USD 3 million to apply each year, fumigation is gradually being phased out because of its negative impacts on fruit quality, the environment and public health. In the early 1990s, working with the Joint FAO/IAEA Programme, the National Plant Protection Organization (SENASA) went in a different direction. It established the National Fruit Fly Control and Eradication Programme (PROCEM), which added an environment-friendly and cost-effective component, the sterile insect technique (SIT), within its pest management plans for Patagonia and Mendoza's Central and South Oases.



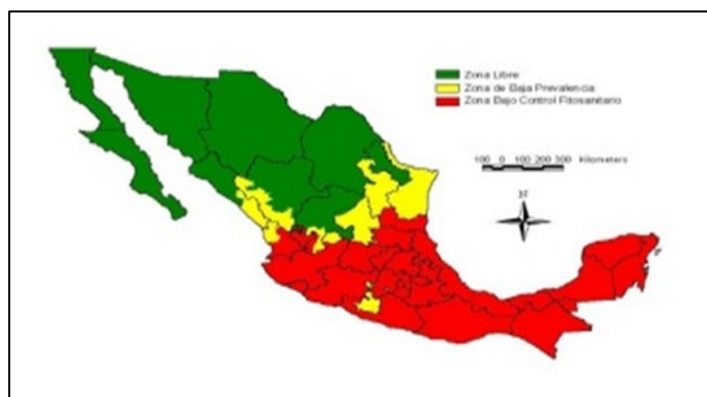
Cherries produced in the Patagonia fruit fly free area (Photo: PROCEM-SENASA Argentina).

The SIT has been used in Argentina since 1992. As a result, parts of Patagonia were declared fruit fly free in 1999, the rest in 2006. Similarly, parts of the South Oasis in Mendoza were declared fruit fly free in 2003, the rest in 2006, and the whole of the Central Oasis in Mendoza was declared fruit fly free in 2004. In 2018 and 2019, China, the world's largest fresh fruit market, verified Patagonia and Mendoza fruit fly free status and agreed that fresh fruits were indeed free of fruit flies, and therefore met the necessary requirements for air freight shipments, and that the post-harvest cold treatment was no longer required. Exports of pome and stone fruits from these areas are valued at hundreds of millions of US dollars per year.

Mexico's National Fruit Fly Campaign (1992–2019)

In 1992 the Mexican federal government approved the National Fruit Fly Campaign of the Plant Protection General Directorate of the Ministry of Agriculture, Livestock and Rural Development (SAGARPA). The National Campaign

operates through state governments and fruit-grower associations under compliance agreements subscribed to by the three parties (federal and state governments, and fruit industry). The federal government facilitates the legal instruments for smooth implementation of the programme, supplies the sterile flies, and operates international quarantine stations at ports of entry. The state governments, through their plant protection infrastructure, are responsible for distributing and releasing the sterile flies, and for conducting field activities outside the fruit production areas to assure area-wide control of the pests. Responsibilities also include operating quarantine interstate road stations. At the farm level, the programme is conducted by the fruit industry, through Plant Protection Committees, that link the producer associations with the state and federal governments. Activities of producers in orchards include trapping, applying bait sprays, and releasing sterile flies.



Fruit fly free (green), low prevalence (yellow) and high prevalence areas (red) in Mexico. (Image: SENASICA Mexico).

For SIT application two modular mass-rearing facilities were built in the town of Metapa de Dominguez, Chiapas, Mexico, for mass-production of the Mexican fruit fly (*A. ludens*) and the West Indies fruit fly (*A. obliqua*), each facility with a production capacity of 150 million sterile flies per week. In the first 4 years after 1997, when fruit fly eradication in north-west Mexico was officially declared, the direct benefits (reduced fruit fly damage and increased yield) amounted to USD 25 million. In addition, in the same time period, the benefits obtained from the price differential paid by export markets, and savings in postharvest treatments, totaled approximately USD 35 million.

Sterile Fly Preventive Release Programme (PRP) in California and Florida (1995–2022)

California and Florida, the main USA horticulture producing states, used a reactive approach to control medfly outbreaks, such as ground and aerial insecticide-bait spraying which was only partially effective and also had negative public health and environmental impacts. In the mid-1990s, at the recommendation of a Technical Advisory Committee in which staff of the IAEA and its Joint FAO/IAEA Programme participated and had a leading role, California and Florida both initiated the sterile insect technique, calling for area-wide preventive and continuous aerial releases of

sterile male medflies over high-risk areas. Setting up a preventive SIT programme and ensuring the weekly availability of millions of sterile medflies to release over the large areas at risk, proved to be a challenge. The solution came from the largest insect rearing facility in the world:



Perimeter of the sterile fly preventive release area over Los Angeles Basin (Photo: CDF).

El Pino facility of the Moscamed Programme in Guatemala. The hundreds of millions of sterile male insects needed to effectively cover the areas at risk are shipped weekly from Guatemala and delivered for release dispersion in the two states. The reactive insecticide-bait approach in California had a direct average cost of approximately US\$33 million a year which included the insecticide and labour costs, but mainly represented market losses due to quarantine restrictions. Whereas the preventive SIT approach, with an overall cost of approximately US\$13 million annually. Not only were expenses cut by more than half, but there were multimillion dollar savings from avoiding restrictions to trade of horticultural products from California and Florida to many countries in the world that are free from this pest.

Eradication of cactus moth from the Yucatan Peninsula in Mexico (2006–2009)

The cactus moth is an invasive species native to South America, including northern Argentina, Uruguay, Paraguay and southern Brazil. It expanded its range to the Caribbean islands and eventually to the Florida Keys off the US Gulf Coast and to mainland Florida. At a later stage, in 2006, it also infested two islands off the Yucatan Peninsula, from which it threatened Mexico's enormous commercial cultivations of *Opuntia* as well as the arid ecosystems of the area that depend on *Opuntia* cactus for soil and wildlife conservation. The threat was enough to bring Mexico's Ministry of Agriculture, the US Department of Agriculture (USDA), the Joint FAO/IAEA Programme and IAEA's Technical Cooperation Department together to implement an eradication programme to eliminate the Yucatan infestation.



Cactus moth Cactoblastis cactorum infesting Opuntia cactus (Photo: Ignacio Baez USDA).

The invasive cactus moth was found in an *Opuntia* cactus on Mujeres and Contoy Islands. The emergency response included surveillance tools and the sterile insect technique. The infested area was defined setting out baited traps within the area, cutting back any infested cactus and eliminating *Opuntia* plants that could act as hosts to the moths, replacing them with ornamental plants. These initial steps served to greatly suppress the moth population on the two islands, after which the campaign employed the sterile insect technique (SIT). This meant bringing to Mexico sterilized moths that had been reared in Florida for the US cactus moth containment campaign. By October 2009, when three life cycles of the pest had passed without any further detection, the cactus moth was officially declared eradicated. This work avoided significant damage to the economically important cactus and prickly pear industries in Mexico, and to the *Opuntia* arid ecosystems.

How a Nuclear Technique Helped Save the Western Cape's Orange Industry (2007–2022)

South Africa is the second largest exporter of fresh citrus fruit in the world, with exports worth over US \$1.4 billion in 2014. Back in 2005, the main export market for the region's citrus fruit, the United States, tightened import quality and infestation reduction measures, as U.S. agriculture grew concerned about the spread of the false codling moth to their country, potentially threatening citrus and cotton industries. Farmers in the area used to lose between 10% and 15% of their production to the pests before harvest, but the real losses came from the pest-infested fruits that made it into shipments and were returned by U.S. inspectors. Following the success of the IAEA-supported SIT trial, the Citrus Growers'



Citrus fruit is the second most important agricultural export commodity in South Africa, with most of the production destined for exports. (Photo: M. Gaspar/IAEA).

Association and the government co-founded XSIT in order to industrialize the use of the technique. Now the association fully owns XSIT, which charges farmers for its services and runs on a fully commercial basis. The area it serves has increased more than ten-fold since 2007, and it has contracts in place to further expand to a total of 21 000 hectares. XSIT produces and releases 40 million sterile moths every week in an area of over 15 000 hectares in the provinces of Western and Eastern Cape. Citrus orchards free of the devastation of false codling moth in the Elephant's River Valley, and an industry, once on the brink of extinction, is now thriving again.

Eradication of a Mediterranean fruit fly outbreak in the Dominican Republic (2015–2017)

The Caribbean Region had always been free of the invasive and destructive Mediterranean fruit fly or medfly until March of 2015, when medfly was introduced and spotted in a lone almond tree in Punta Cana located on the eastern region of the Dominican Republic. In the initial nine months Dominican producers had already lost 40 million dollars due to their inability to export to the USA.



Plaque presented by FAO and IAEA to the Minister of Agriculture of the Dominican Republic in recognition to his commitment and leadership in the successful Mediterranean fruit fly eradication campaign (Photo: IAEA).

The national authorities requested support in setting up a country-wide surveillance network and an eradication campaign. Immediate support was provided by FAO and IAEA through their Joint FAO/IAEA Programme and the IAEA's Technical Cooperation Department, as well as by the US Department of Agriculture (USDA) and the Guatemala-Mexico-USA Moscamed Programme. Other organizations, including Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) and the Instituto Interamericano de Cooperación para la Agricultura (IICA) also provided valuable support.

The campaign identified an infestation of more than 2 000 km². First step was to suppress the medfly population, using ground bait sprays and bait stations placed on trees. Almond trees that had hosted the first indication of medflies were heavily pruned. SIT was used as the final step of the medfly eradication process – a clean-up operation to eliminate any populations that remained. Starting in October 2015 and ending in April 2017, each week up to 82 million sterilized flies were shipped to the Dominican Republic from their rearing facility in Guatemala. The campaign went so well that in January 2016, trade reopened with the USA, while the SIT eradication was still underway. Eradication was officially declared by the Ministry of Agriculture in July 2017.

Mediterranean Fruit Fly Suppression by National Action Plan in Neretva River Valley of Croatia (2017–2018)

Citrus cultivation is very important in Croatian fruit growing. 75% of their total mandarin production is being exported. Due to huge economic damage caused by the Mediterranean fruit fly, in 2017 the Minister of Agriculture adopted the Action Plan for the Prevention of Spreading and Suppression of the Mediterranean Fruit Fly in the Neretva Valley. Activities carried out on 6 000 ha of the valley area include: monitoring of adult and larval populations, early detection of hotspots and response in case of fruit fly presence, supervision of implementation of sanitary measures defined by the law, implementation of suppression measures and mass-trapping in peach or nectarines orchards, natural environment and the public areas, the removal of individual uncultivated host trees on public areas and uncultivated orchards, as well as education of farmers.

Apart from these activities, a total of 4 000 ha (of the 6 000 ha) in the area from the Neretva Estuary to Opuzen, is also being treated with sterile male Mediterranean fruit flies. In the year 2017, a maximum FTD of 0.008 was recorded for the Neretva estuary/Opuzen area, an area where all available control tactics have been implemented. In the Opuzen-Metković area where the SIT has so far not been applied, the highest FTD recorded was 0.5. In 2018, the highest FTDs were 0.049 and 0.341 for the Neretva estuary/Opuzen and the Opuzen-Metković area, respectively. According to the Phytosanitary Inspection Report, none of the exported

shipments of mandarins originating from Neretva has been returned due to the presence of larvae. These actions have resulted in a successful suppression of the Mediterranean fruit fly in the Neretva Valley.



Citrus growing area in Neretva River valley, Croatia (Photo: IAEA).

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 in Ecuador (2018–2020)

Ecuador, one of the largest producers of tropical fruit in the Western Hemisphere, is adding non-traditional fruits to its export portfolio as a result of successfully fighting off the Mediterranean fruit fly with the help of nuclear techniques. After receiving technical assistance from the IAEA, in partnership with the Food and Agriculture Organization of the United Nations (FAO), to use – along with other methods – the sterile insect technique (SIT) to control the flies, farmers are now shipping golden berries, dragon fruit and tree tomatoes to markets in the United States, Latin America and the European Union.



Tree tomato being harvested in Ecuador that is now exported to markets in the United States, Latin America and the European Union. (Photo: AGROCALIDAD).

As a result, in 2019 exports to the United States of these three fruits reached USD 22 million. In addition, the

agricultural sector benefited from direct and indirect jobs that have been created along the production and export chain. Three million sterilized fruit flies are shipped weekly from the El Pino fruit fly facility, Guatemala, to continental Ecuador where they are kept at a sterile fly emergence and release facility built under the auspices of an IAEA technical cooperation project. They are then released in fields over targeted production areas where IAEA trained agronomists from AGROCALIDAD monitor and control the fruit fly populations. Ecuador is now seeking to expand the use of the SIT technology to other areas and other crops.

Eradication of the Mediterranean Fruit Fly Outbreak in Colima Mexico (2021–2022)

An extensive outbreak of the Mediterranean fruit fly or medfly, one of the most devastating insect pests infesting fruits and vegetables, has been successfully eradicated in the Mexican state of Colima, as announced by the Mexican National Service for Agrifood Health, Safety and Quality (SENASICA) in August 2022. In cooperation with the International Atomic Energy Agency (IAEA) and the Food and Agricultural Organization of the United Nations (FAO), Mexico used the sterile insect technique (SIT) to eradicate the medfly that had been threatening fruit and vegetable crops, farmers' livelihoods and the country's economy.



FAO/IAEA Technical Advisory Panel (TAP) visiting the Port of Manzanillo in Colima, Mexico, April 2022 (Photo: SENASICA Mexico).

The Colima outbreak, detected in April 2021 in the country's largest port, Manzanillo, posed an immediate risk to crops such as carambolas, figs, guavas, mangoes, papaya, pink grapefruits and oranges. If not managed promptly, Mexico, the world's seventh-largest producer and exporter of fresh fruits and vegetables, could face quarantine restrictions imposed by countries free from this pest. This could affect the country's trade in such goods, which generates over 189 billion Mexican pesos (EUR 8.8 billion) annually in exports, as well as millions of local jobs.

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

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 Kouri’ (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.



Equipment for the mass-rearing of Aedes aegypti installed at the Institute of Tropical Medicine ‘Pedro Kouri’ (Photo: Rene Gato).

The mosquitoes were reared in a small facility located at the IPK. The male insects were sterilized as pupae in a Gammacell 2020 installed at CEADEN. A monitoring network with ovitraps was maintained in collaboration with the local populations. 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. Since then, around 40 000 mosquitoes have been re-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.

II. Research and Development

Inauguration of the New Insect Pest Control Laboratory (IPCL) in Seibersdorf, Austria

A very important event for the future of the Insect Pest Control Section was the inauguration on 25 of September 2017 of the new Insect Pest Control Laboratory (IPCL) in Seibersdorf, Austria. The new laboratory, with over 1,700 m² of laboratory space, will become operative during 2018, after gradually transferring the >250 insect species, strains and populations currently maintained at the old laboratory – a unique repository that Member States can draw upon. It will substantially increase the ability to assist Member States in applying nuclear techniques to combat major insect pests that spread diseases and damage crops. The new building has more and better-quality space and equipment to improve work-flow and efficiency and output. The inauguration marks a milestone in the IAEA’s initiative to modernise its eight nuclear applications laboratories, built in Seibersdorf in 1962, under a project known as ReNuAL. Along with additional space to train experts to support the transfer of SIT to countries, the new IPCL will facilitate research on development and application of more environmentally friendly and therefore more sustainable pest control techniques. The new building will also feature a unique 230 m² ‘Ecosphere’ – a climate-controlled facility – to help scientists study the behaviour of sterilised insects.



New FAO/IAEA Insect Pest Control Laboratory in Seibersdorf, Austria, inaugurated on 25 September 2017.

The Development of the Mediterranean Fruit Fly Genetic Sexing Strains

In 1979, Yoram Rössler constructed a strain of the Mediterranean fruit fly in which male pupae (brown) could be separated from female pupae (white). This special strain was mass-reared, and pupae sorted by a seed-sorter at the FAO/IAEA Seibersdorf Laboratory in Austria and was it performed well in large-scale tests in Israel.

Subsequently, this laboratory developed a genetic sexing strain in which a segment of an autosome bearing the dominant wild type allele of a temperature-sensitive lethal

(*tsl*) mutant was translocated to the Y chromosome. This enabled the elimination of females at the egg stage, thereby saving significant costs during mass-rearing, handling, and release, and increasing sterile male production in the same facility.



VIENNA 8 a Mediterranean fruit fly genetic sexing strain (GSS) developed over the years at the insect pest control laboratory and that contains two mutations, the white pupae (*wp+*) and the temperature-sensitive lethal (*tsl*).

Significant savings can be gained if females are eliminated early in the rearing process. The role model for sexing is the Mediterranean fruit fly *tsl* strain developed by classical genetic approaches, where the *tsl* mutation specifically and effectively kills all female embryos upon heat shock.

In addition, a “filter rearing system” to maintain strain stability in the mass-rearing of such genetic sexing strains was developed.

Development of Improved Attractants and Their Integration into Fruit Fly SIT Management Programmes

Different trap, lures and bait stations were tested in a wide range of environmental conditions to measure the response of a number of fruit fly species of economic importance. As a result of a five-year Research Coordinated Project important progress was made in improving some of the trapping systems currently being used against these fruit fly species. In general, response of some of the most important *Anastrepha* species to a two-component synthetic food lure was equal or better compared with the conventional but less practical protein baits. Likewise, the *Bactrocera* species responded much better to combinations and different concentrations of the synthetic food lures than to the conventional protein baits.

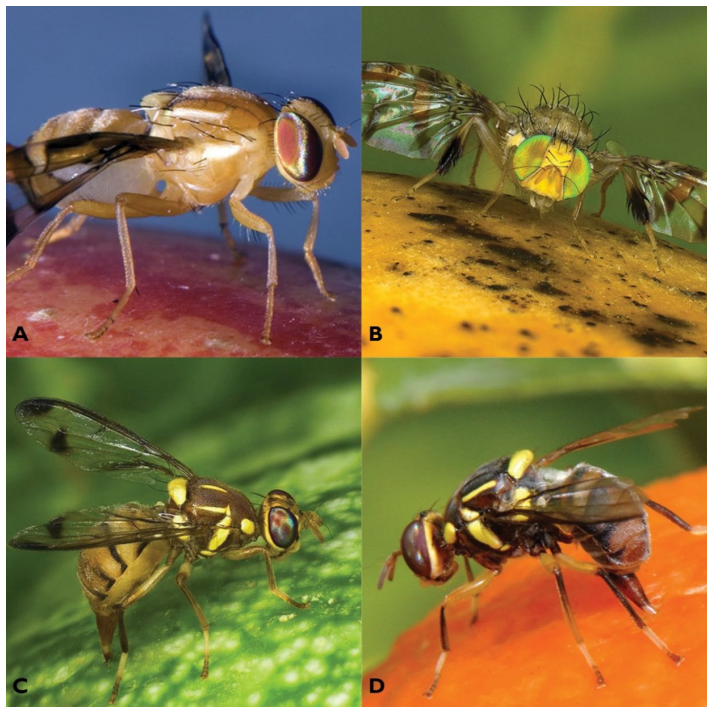


Multilure trap used for a wide range of attractants and fruit fly species (Photo: IAEA).

It was observed that the level of female response of some species of *Ceratitidis*, in particular, *C. rosa* and *C. cosyra*, to the female biased three component lure (Biolure), is the same as *C. capitata*. On the other hand, species which are more host specific such as the Guava Fruit Fly, (*A. striata*) and the Olive Fruit Fly (*B. oleae*) had poor response to the synthetic food lures as compared with the conventional protein baits. This research resulted in the development of the now commercially available female biased food synthetic lure (Biolure with three and two components), being widely used for surveillance of *C. capitata* and several species of *Anastrepha* including *A. ludens*, *A. obliqua* and *A. suspensa*. Moreover, it also resulted in the development of two novel traps, namely the Multilure trap (dry and liquid trap) and the Fase-IV trap (dry trap), both used in combination with the Biolure.

Resolution of Cryptic Species Complexes of Tephritid Pests

In 2015 we concluded the six-year Coordinated Re-search Project (CRP) on “Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade”. The objective of the CRP was to undertake targeted research into the systematics and diagnostics of taxonomically challenging fruit fly groups of economic importance. One of the scientific outputs of the CRP was the accurate alignment of some biological species with taxonomic names.



Images of representatives of each the four tephritid fruit fly cryptic species complexes studied to delimit species boundaries to overcome constraints to Sterile Insect Technique (SIT) application and international trade. A *Anastrepha fraterculus*, B *Ceratitis rosa* (R2 type), C *Zeugodacus cucurbitae*, D *Bactrocera dorsalis* (Photo credits: A Michal Hoskovec, B and C Antoine Franck, D Ana Rodriguez).

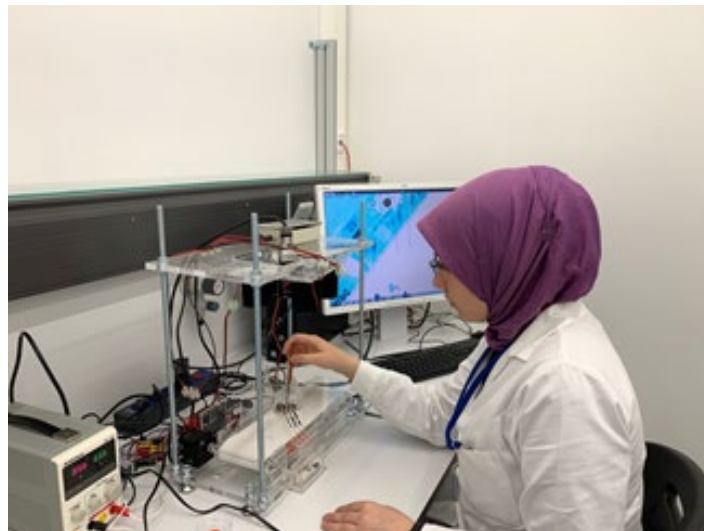
The resolution of some of these controversial issues has important applied implications for FAO and IAEA Member States, both in overcoming technical constraints to the application of the Sterile Insect Technique (SIT) against pest fruit flies and in facilitating international agricultural trade. A Special Issue in the peer-reviewed journal *ZooKeys* [http://zookeys.pensoft.net/browse_journal_issue_documents.php?issue_id=763] has been published, with 26 research and review articles.

The Development and Evaluation of a Near Infrared Pupal Sex Sorter (NIRPSS) in Support of Tsetse Programmes

For more than 15 years, attempts have been made to develop methods to separate the sexes of tsetse pupae a few days before adult emergence. Different versions of “near infrared sex sorter devices” have been developed and tested, but the results were not consistent and satisfactory. The finding in 2016 that wing melanization inside the puparium can be made visible using near infrared was a breakthrough, as this phenomenon can be detected a few days earlier in females than males. The Near Infrared Pupal Sex Sorter (NIRPSS) device that was developed, allows the scrolling of the pupae whilst making several image frames for each pupa at different positions. This makes the detection of melanized wings much more accurate.

As the melanization process is progressing with pupal age, the most suitable pupal age for the sorting was determined. In addition, several parameters were tested to improve the

accuracy of the sex separation. So far, an accuracy of 91% of males was found in un-melanized pupae (assumed to be males) sorted by the NIRPSS. It is important to note that the development rate of the pupae is affected by temperature and relative humidity. Therefore, having a relative constant environmental condition and synchronized pupal age will improve the accuracy of the NIRPSS machine in separating the sexes of the pupae.



Ms Sumejja Canic sorting male and female tsetse pupae using the near infrared sex pupal sorting machine.

In addition, test shipments of male *G. p. gambiensis* pupae sorted with the NIRPSS system at the IPCL and shipped to Dakar, Senegal have been initiated. Results indicate more than 70% operational males.

The development of the sterile insect technique (SIT) for disease transmitting mosquitoes

In response to this demand, the Joint FAO/IAEA Programme increased efforts towards the full development and improvement of the SIT package for the area-wide management of mosquitoes to support its Member States. This was done through a significant extrabudgetary contribution of some Member States in support of research and development activities. Collaborators have also intensified their research and implementation of SIT in pilot projects against human disease vectors. Therefore, the recent progress made on the development of the SIT package for mosquito vectors and the status of transfer of the existing technology in view of the continuous requests by the Member States are the main drivers for reviewing a Thematic Plan.



A sterile male *Aedes albopictus*. (Photo: T. Wallner FAO/IAEA).

The SIT is not a stand-alone technology. To be effective, it should be integrated in a package with non-nuclear techniques (biological, chemical, behavioural) while economic considerations and public education should also be considered to secure support and promote further implementation. Ideally, SIT should be part of an Area-Wide Integrated Pest Management Approach in which the total population of a pest or a disease vector in a region is managed.

III. Publications

“Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management” and “Area-Wide Integrated Pest Management: Development and Field Application”

The second edition of the book ‘Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management’ takes a generic, thematic, comprehensive and global approach in describing the principles and practice of the sterile insect technique (SIT). The major chapters discuss the principles and technical components of applying sterile insects. Other chapters deal with supportive technologies, economic, environmental and management considerations, and the socioeconomic impact of area-wide integrated pest management (AW-IPM) programmes that integrate the SIT.

The book ‘Area-Wide Integrated Pest Management: Development and Field Application’ deals with all aspects of the application of AW-IPM approaches that aim at the management of total pest populations, involving a coordinated effort over often larger areas. For major livestock pests, vectors of human diseases and pests of high value crops with low pest tolerance, there are compelling economic reasons for implementing AW-IPM programmes.



Front cover of the second edition of ‘Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management’ (<https://doi.org/10.1201/9781003035572>) (left) and ‘Area-Wide Integrated Pest Management: Development and Field Application’ (<https://doi.org/10.1201/9781003169239>) (right).

Reorganization and Harmonization of International Fruit Fly Standards

The Commission on Phytosanitary Measures (CPM) of the International Plant Protection Convention (IPPC), acknowledged the Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies (TPFF) for its work towards the preparation of the proposal. The main objective was to re-organize and harmonize the existing suite of fruit fly standards (International Standard for Phytosanitary Measures or ISPMs) so that they are more logical, simplifying their implementation to facilitate agricultural trade and prevent the introduction and spread of invasive fruit flies.

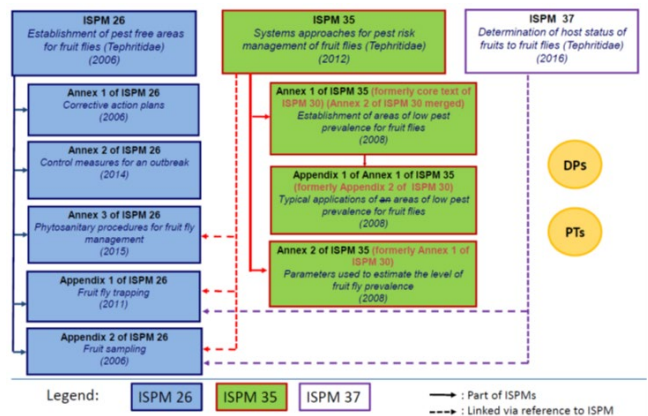
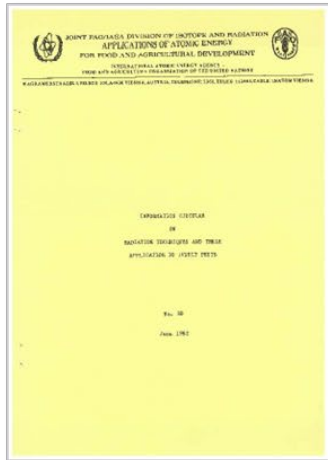
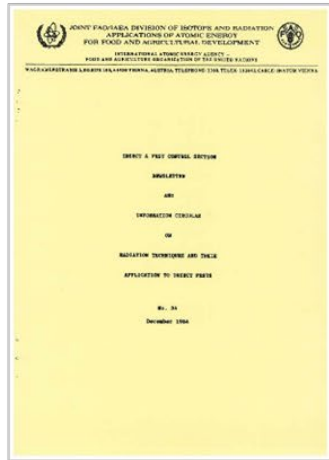


Chart showing the reorganization of fruit fly international standards.

40 Years of the IPC Newsletter: From an Informal Circular to a Formal Publication



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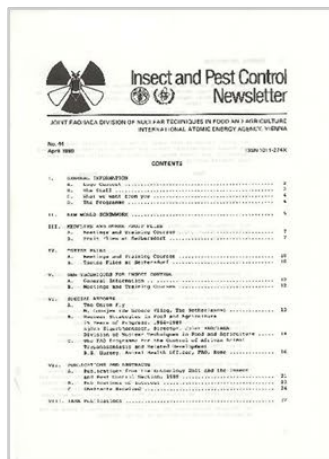
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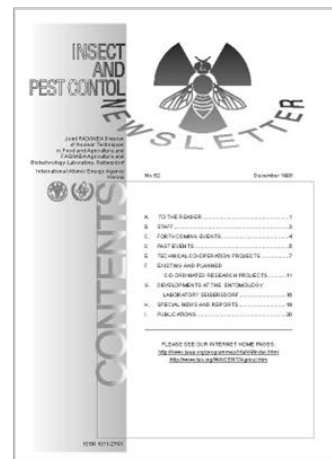
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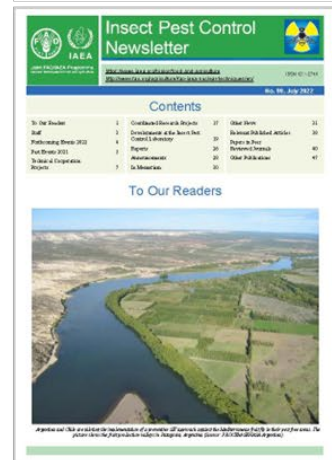
1998



2004



2014



2022

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Forthcoming Events (2023)

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

Third RCM on Improvement of Colony Management in Insect Mass-rearing for SIT Applications. 27 February–3 March 2023 (virtual).

Third RCM on Generic Approach for the Development of Genetic Sexing Strains for SIT Applications. 24–28 April 2023 (virtual).

Second RCM on Improving Rearing, Handling, and Field Components for Fruit Fly SIT Application. 15–19 May 2023, Vienna, Austria.

First RCM on Mosquito Male Performance. 3–7 July 2023, Vienna, Austria.

Third RCM on Mosquito Irradiation, Sterilization and Quality Control. 6–10 November 2023 (virtual).

II. Consultants and Expert Meetings

FAO/IAEA Consultancy Meeting on Thematic Plan for Lepidoptera Sterile Insect Technique. 6–10 February 2023, Vienna, Austria.

FAO/IAEA Consultancy Meeting on *Drosophila suzukii*–New Other Species for SIT. 27 February–3 March 2023 (virtual).

FAO/IAEA Consultancy Meeting on Thematic Plan for Tsetse Area-wide Integrated Pest Management Programmes Involving the Sterile Insect Technique. 29 May–2 June 2023, Vienna, Austria.

III. Other Meetings/Events

FAO/IAEA Workshop on Genetic Diversity Analysis and Colony Management. 23–25 February 2023, Patras, Greece.

FAO/IAEA Regional Training Course on Genetic Population Studies to Support Tsetse Field Projects (under Regional TC Project RAF5087). 17–28 April 2023, Kenya. FAO/IAEA Regional Group Scientific Visit on Area-Wide Eradication Programmes of the New World Screwworm (under Regional TC Project RLA5088). 3–7 March 2023, Pecora, Panama.

FAO/IAEA Regional Meeting on Genetics, Breeding Procedures and Other Characteristics of the New Genetic Sexing Strain of *Anastrepha fraterculus* (Wiedmann) (under Regional TC Project RLA5087). 27–31 March 2023, Mendoza, Argentina.

FAO/IAEA Regional Training Course on Applying State of the Art Dosimetry and Quality Control Tests for South American fruit fly *Anastrepha fraterculus* Wied. (under Regional TC Project RLA5087). 17–21 April 2023, Siebersdorf, Austria.

FAO/IAEA Coordination Meeting on Enhancing Capacity for the Use of the Sterile Insect Technique as a Component of Mosquito Control Programs (under Regional TC Project RLA5083). 12–16 June 2023, Havana, Cuba.

FAO/IAEA Regional Training Course on Fruit Fly Surveillance and Identification (under Regional TC Project RAS5097). 10–14 July 2023, Kuala Lumpur, Malaysia.

FAO/IAEA Regional Training Course on Collection of Entomological Baseline Data, and Data Management Tools to Support SIT Projects for Mosquito Control (under Regional TC Project RLA5083). 10–21 July 2023, Juazeiro, Bahia, Brazil

International Congresses of Dipterology (ICDX). 16–21 July 2023, Reno, Nevada, USA

FAO/IAEA Interregional Training Course on Dosimetry and Irradiation Procedures to Support SIT Field Projects for Vector Control (under Regional TC Project RLA5083, RER5026, RAS5095, RAF5087). 2–6 October 2023, Vienna, Austria.

Past Events (2022)

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

Second RCM on Mosquito Irradiation, Sterilization and Quality Control. 18–22 July 2022, Vienna, Austria.

First RCM on Improve the Mass-Rearing of Lepidoptera Pests for SIT Programmes. 5–9 September 2022, Vienna, Austria.

Fourth RCM on Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management. 8–12 November 2022, Sydney, Australia.

Third RCM on Assessment of Simultaneous Application of SIT and MAT to Enhance *Bactrocera* Fruit Fly Management. 20–24 November 2022, Sydney, Australia.

II. Consultants and Expert Meetings

FAO/IAEA Consultancy Meeting on Mosquito Male Performance. 23–27 May 2022, Vienna, Austria.

FAO/IAEA Consultancy Meeting on *Aedes* Mosquito Sterile Insect Technique Package: Review and Future Perspectives. 30 May–1 June 2022, Vienna, Austria.

FAO/IAEA Consultancy Meeting on Guideline on Transboundary Shipments of Sterile Insects. 13–17 June 2022, Vienna, Austria.

III. Other Meetings/Events

FAO/IAEA Regional Workshop on Challenges and Solutions in the Implementation of SIT based Technologies against *Aedes* Vectors (under Regional TC Project RAS5082), co-organized with the National Environment Agency, Singapore. 12–19 January 2022 (virtual).

FAO/IAEA Second Coordination Meeting on Enhancing Capacity for the Use of the Sterile Insect Technique as a Component of Mosquito Control Programmes (under Regional TC Project RLA5083). 1–3 February 2022 (virtual).

FAO/IAEA Regional Training Course on Pest Risk Analysis (under regional TC Project RLA5090). 23–29 March 2022 (virtual).

FAO/IAEA Regional Stakeholder Engagement Meeting to Achieve Early Support to Sterile Insect Technique Pilot Trials against *Aedes* Mosquitoes (under Regional TC Project RER5026). 4–6 April 2022, Bologna, Italy.

FAO/IAEA Regional Training Course on Vector Control Needs Assessment. Co-organized with the World Health Organization Regional Office for Europe (under Regional TC Project RER5026). 9–13 May 2022, Limassol, Cyprus.

FAO/IAEA First Coordination Meeting on Validating the Sterile Insect Technique for the Control of the South American Fruit Fly (under Regional TC Project RLA5087). 23–25 May 2022 (virtual).

FAO/IAEA First Coordination Meeting on Advancing Surveillance and Progressive Control of the New World Screwworm Using the Sterile Insect Technique (under Regional TC Project RLA5088). 1–3 June 2022 (virtual).

FAO/IAEA First Coordination Meeting on Enhancing Regional Capacity for the Implementation of the Sterile Insect Technique as a Component for Area-Wide Tsetse and Trypanosomosis Management (under Regional TC Project RAF5087). 20–24 June 2022, Vienna, Austria.

FAO/IAEA First Regional Coordination Meeting on Strengthening and Harmonizing Surveillance and Suppression of Fruit Flies (under Regional TC Project RAS5097). 21–23 June 2022 (virtual).

FAO/IAEA High-Level Group Scientific Visit for Uruguay on Area-Wide Integrated Pest Management Programmes for the Control of the New World Screwworm (under Regional TC Project RLA5088). 27 June–1 July 2022, Pecora, Panama.

FAO/IAEA Workshop on Irradiation and Dosimetry. 14–15 July 2022, Vienna, Austria.

XXVI International Congress of Entomology. 17–22 July 2022, Helsinki, Finland.

FAO/IAEA Regional Training Course on Mark-Release-Recapture and Field Data Management (under Regional TC Project RAS5095). 29 August–2 September 2022, Vienna, Austria.

FAO/IAEA Regional Training Course on Identification of the South American Fruit Fly (*Anastrepha fraterculus*) Morphotypes, Management of the Genetic Sexing Strain Colonies, and Artificial Rearing (under Regional TC Project RLA5087). 5–9 September 2022, Seibersdorf, Austria.

Meeting of the Technical Panel on Phytosanitary Treatments (TPPT), International Plant Protection Convention FAO. 12–16 September 2022, Rome, Italy.

First International Plant Health Conference. 21–23 September 2022, London, UK.

FAO/IAEA Training Course on Codling Moth IPM Integrating SIT (under National TC Project CPR5027). 10–19 October 2022 (virtual).

Horizon 2020 Project, Controlling and Progressively Minimizing the Burden of Animal Trypanosomosis (COMBAT) First Annual Meeting. 19–21 October 2022, Montpellier, France.

FAO/IAEA Project Coordination Meeting and Regional Workshop on Phased Conditional Approach (under Regional TC Project RAS5095). 7–11 November 2022, Jakarta, Indonesia.

FAO/IAEA Regional Training Course on SIT Components: Handling, Shipping and Releasing Procedures Applied in SIT Projects (under Regional TC Project RLA5083). 7–11 November 2022, Juazeiro, Brazil.

11th International Symposium on Fruit Flies of Economic Importance. 14–18 November 2022, Sydney, Australia.

FAO/IAEA Taskforce Meeting on Strengthening PATTEC activities in Africa (under regional TC Project RAF5087). 15–18 November 2022, Vienna, Austria.

FAO/IAEA Regional Training Course on Methods for the Mass-Rearing, Irradiation and Release of Sterile Male *Aedes* spp. (under Regional TC Project RAS5095). 21–25 November 2022, Singapore, Singapore.

FAO/IAEA Mid-term Review Meeting on Enhancing the Capacity to Integrate Sterile Insect Technique in the Effective Management of Invasive *Aedes* mosquitoes (under Regional TC Project RER5026). 28 November–02 December 2022, Pula, Croatia.

FAO/IAEA Regional Training Course on Tsetse Baseline Data Collection and Data Management (under regional TC Project RAF5087). 28 November–2 December 2022, Harare, Zimbabwe.

FAO/IAEA Regional Training Course on New World Screwworm Identification and Diagnosis (under regional TC Project RLA5088). 5–9 December 2022, Montevideo, Uruguay.

FAO-WHO/PAAT Expert Meeting on Indicators of Vector Control coverage in gHAT elimination. 14–15 December 2022, Rome, Italy.

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	Ongoing National Projects	Technical Officer
Bangladesh	BGD5035	Validating the Sterile Insect Technique as a Key Component of an Area-Wide Integrated Pest Management Programme Against <i>Aedes aegypti</i> in Dhaka	Maylen Gómez
Bolivia	BOL5023	Fruit Fly Control in Bolivia Using Integrated Pest Management Including 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)	Rui Cardoso Pereira
Burkina Faso	BKF5023	Implementing the Sterile Insect Technique to Reduce Wild Populations of <i>Aedes aegypti</i> and Tsetse	Adly Abdalla Maylen Gómez
Cameroon	CMR5026	Supporting the National Fruit Fly Management Programme	Daguang Lu
Cambodia	KAM5006	Implementing Fruit Fly Surveillance and Control Using Area-wide Integrated Pest Management	Daguang Lu
Chad	CHD5011	Implementing the Sterile Insect Technique to Control <i>Glossina fuscipes fuscipes</i> — Phase II	Adly Abdalla 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
China	CPR5027	Demonstrating Feasibility of the Sterile Insect Technique in the Control of the Codling Moth, <i>Cydia pomonella</i>	Walther Enkerlin
Cuba	CUB5021	Demonstrating the Feasibility of the Sterile Insect Technique in the Control of Vectors and Pests	Rui Cardoso Pereira
Cyprus	CYP5020	Developing a National Rapid Response Strategy for the Prevention of the Establishment of the Asian Tiger Mosquito	Jeremy Bouyer
Dominican Republic	DOM0006	Building and Strengthening the National Capacities and Providing 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 Release of <i>Aedes aegypti</i> and <i>Philornis downsi</i> Males	Maylen Gómez Walther Enkerlin
Ethiopia	ETH5023	Enhancing Livestock and Crop Production through Consolidated and Sustainable Control of Tsetse and Trypanosomosis to Contribute to Food Security	Chantel de Beer
El Salvador	ELS5015	Integrated Management of Fruit Flies using the Sterile Insect Technique to Establish Areas of Low Prevalence of Fruit Flies	Walther Enkerlin
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 Agricultural Pests Using Nuclear Technologies	Walther Enkerlin
Israel	ISR5022	Establishing the Sterile Insect Technique Methodology for the Management of the False Codling Moth, <i>Thaumototibia leucotreta</i> , and Enhancing Integrated Pest Management Against the Peach Fruit Fly, <i>Bactrocera zonata</i>	Walther Enkerlin
Jamaica	JAM5014	Establishing a Self-Contained Gamma Irradiation Facility for the Introduction of Sterile Insect Technique and Experimental Mutagenesis and Diagnostic Technologies	Rui Cardoso Pereira
Libya	LIB5014	Supporting Control of Fruit Flies by Establishing a Low Fruit Fly Prevalence Zone	Daguang Lu
Mauritius	MAR5028	Enhancing National Capabilities on the Suppression of <i>Aedes albopictus</i> in an Urban Locality Using the Sterile Insect Technique as Part of an 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
Myanmar	MYA5029	Improving Fruit Yield and Quality by Using Sterile Insect Techniques as Part of Area-Wide Integrated Pest Management of Fruit Flies in the Mandalay Region	Daguang Lu
Palau	PLW5003	Facilitating Sustainability and Ensuring Continuity of Area-wide Pest Management — Phase III	Daguang Lu
Portugal	POR5006	Integrating the Sterile Insect Technique in the Control of the Invasive Vector Mosquito <i>Aedes albopictus</i>	Maylen Gómez
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 Area-wide Integrated Pest Management Approach with a Sterile Insect 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	SUD5042	Implementing the Sterile Insect Technique for Integrated Control of <i>Anopheles arabiensis</i> — Phase III	Adly Abdalla
Turkey	TUR5026	Conducting a Pilot Program on Integrated Management of <i>Aedes aegypti</i> Including Sterile Insect Technique	Maylen Gómez
Turkey	TUR5027	Implementation of SIT for Suppression and Eradication of Medfly in Turkey	Daguang Lu
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
Ongoing 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	RAF5087	Enhancing Regional Capacity for the Implementation of the Sterile Insect Technique as a Component for Area-Wide Tsetse and Trypanosomosis Management (AFRA)	Maylen Gómez
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 Asia & the Pacific	RAS5095	Enhancing the Capacity and the Utilization of the Sterile Insect Technique for <i>Aedes</i> Mosquito Control	Marc Vreysen
Regional Asia & the Pacific	RAS5097	Strengthening and Harmonizing Surveillance and Suppression of Fruit Flies	Daguang Lu Rui Cardoso Pereira
Regional Europe	RER5026	Enhancing the Capacity to Integrate Sterile Insect Technique in the Effective Management of Invasive <i>Aedes</i> Mosquitoes	Wadaka Mamai Jeremy Bouyer
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 Programmes	Maylen Gómez
Regional Latin America	RLA5084	Developing Human Resources and Building Capacity of Member States in the Application of Nuclear Technology to Agriculture	Walther Enkerlin Rui Cardoso Pereira
Regional Latin America	RLA5087	Validating the Sterile Insect Technique for the Control of the South American Fruit Fly (ARCAL)	Walther Enkerlin
Regional Latin America	RLA5088	Advancing Surveillance and Progressive Control of the New World Screwworm Using the Sterile Insect Technique	Walther Enkerlin

Highlights of Technical Cooperation Projects

Strengthening Food Security through Efficient Pest Management Schemes Implementing the Sterile Insect Technique as a Control Method (RLA5082)

Mexico Officially Declared the Eradication of an Extensive Outbreak of the Mediterranean Fruit Fly (*Ceratitis capitata* Wied.) from the State of Colima

The Mediterranean fruit fly (*Ceratitis capitata*), one of the most devastating insect pests infesting fruits and vegetables, has been successfully eradicated in the Mexican state of Colima, as officially announced on 2 August 2022 by the Secretary of Agriculture of Mexico. In cooperation with the International Atomic Energy Agency (IAEA) and the Food and Agricultural Organization of the United Nations (FAO), Mexico used an area-wide integrated pest management that include the sterile insect technique (SIT) to eradicate the Mediterranean fruit fly that had been threatening fruit and vegetable crops, farmers' livelihoods and the country's economy.



The Secretary of Agriculture of Mexico officially declared eradication of the Mediterranean fruit fly outbreak from the state of Colima.

The Colima outbreak, detected in April 2021 in the country's largest port, Manzanillo, posed an immediate risk to crops such as carambolas, figs, guavas, mangoes, papaya, pink grapefruits and oranges. If not managed promptly, Mexico, the world's seventh-largest producer and exporter of fresh fruits and vegetables, could face quarantine restrictions imposed by countries free from this pest. This could affect the country's trade in such goods, which generates over 189 billion Mexican pesos (8.8 billion Euro) annually in exports, as well as millions of local and rural jobs.

After receiving an emergency request for assistance in April 2021, the IAEA and FAO acted immediately, dispatching an expert to help plan, implement and evaluate the eradication actions. This is one more example where SIT has been successfully used to suppress and eradicate invasive insect

pests, contributing worldwide food security and safety. As first action 4 436 traps were deployed for pest delimitation purposes.

To control the outbreak, Mexico designed and implemented an emergency action plan with the assistance of FAO/IAEA experts. Based on this plan, Mexico released more than 1 450 million sterile male Mediterranean fruit flies in Colima.

The sterile male Mediterranean fruit flies were produced at a newly built facility in Metapa de Dominguez, Chiapas, Mexico. The facility, with a design that benefited from FAO/IAEA expertise, was inaugurated in 2021. It is the second largest in the world with a production capacity of 1 000 million sterile medflies every week.

"Mexico has managed to maintain its status as a country free of the Mediterranean fly," said Francisco Ramírez y Ramírez, General Director of Plant Health of the National Service for Agrifood Health, Safety and Quality (SENASICA) of Mexico at the event declaring the eradication of the pest in the State of Colima. "If the Mediterranean fruit fly had settled in the country, the consequence would have been the closure of access to national and international markets for Mexican fruits and vegetables, which ultimately would have meant economic losses for local producers," he added.

The IAEA will continue assisting and working together with Mexico through national and regional technical cooperation projects, and through Mexico's National Fruit Fly Programme (PNMF), an IAEA Collaborating Centre.

Validating the Sterile Insect Technique for the Control of the South American Fruit Fly (RLA5087)

FAO/IAEA Training Course on South American Fruit Fly *Anastrepha fraterculus* Taxonomy and Artificial Rearing. 5–9 September 2022, Vienna Austria



*Participants of the training course on South American Fruit Fly *Anastrepha fraterculus* Taxonomy and Artificial Rearing, 5–9 September 2022, Vienna Austria.*

The training course was attended by seven fellows from Argentina, Brazil, Ecuador, Paraguay, Peru and Venezuela. A total of 35 hours of lectures including theory and practical exercises were offered. The topics covered taxonomy of South American fruit fly *Anastrepha fraterculus*, aspects of *A. fraterculus* morphotypes, colony management, artificial rearing, pupae marking and packing before irradiation, principles of gamma and X-ray irradiation and basic quality control parameters. Hands-on activities included practical exercise on *A. fraterculus* adult and immature identification as well as diet preparation, placement of diet in trays and egg seeding.



Hands-on training on diet preparation.

This event is part of the activities aimed at capacity building for future application of area-wide sterile insect technique (AW-SIT) against the South American fruit fly in Latin America and the Caribbean.

Enhancing the Capacity and the Utilization of the Sterile Insect Technique for Aedes Mosquito Control (RAS5095)

FAO/IAEA Regional Training Course on Mark-Release-Recapture and Field Data Management. 29 August–2 September 2022. Vienna, Austria

This regional TC project aims to equip the participating Member States with technologies, tools and expertise required for implementing SIT-based approaches to suppress the mosquito populations of *Aedes aegypti* and *Aedes albopictus*. To achieve this objective, the project

provides assistance on the capacity development in mass-rearing, irradiation, sterile male releases and field data management, as components of the SIT package for the control of mosquito, the vector of diseases such as chikungunya, dengue and Zika. Moreover, mark-release recapture (MRR) studies are a prerequisite for effectively planning and conducting a SIT pilot trials. Therefore, the appropriate collection, management, and analysis of the data is important to implement adaptive management and warrant the success of male release programs. In this regard, a regional training course on Mark-Release-Recapture and Field Data Management was held with 17 participants from Bangladesh, Indonesia, Iran, Malaysia, Pakistan, Philippines, Singapore, Sri-Lanka, Thailand, and Vietnam.



Participants of the Regional Training Course on Mark-Release-Recapture and Field Data Management. 29 August–2 September 2022. Vienna, Austria.

The training aimed to provide an understanding of the basics behind MRR studies and the main entomological parameters to be measured to gain knowledge on data collection, management, and statistical analysis from MRR and SIT pilot trial data studies of *Aedes* mosquitoes using the custom-made R package ‘sit’ and template spreadsheets.

The knowledge gained by each participant will help to accelerate the development of SIT-based technologies in the respective Member States.

Enhancing Regional Capacity for the Implementation of the Sterile Insect Technique as a Component for Area-wide Tsetse and Trypanosomosis Management (RAF5087)

First Coordination Meeting. 20–24 June 2022, Vienna, Austria

Tsetse flies (*Glossina* spp.) are the vector of African trypanosomes (AT) in sub-Saharan Africa. These Trypanosoma parasites cause sleeping sickness in humans and nagana in cattle. Tsetse flies are present in thirty-six sub-Saharan African countries, and thus they are at risk of these

diseases that affect human health and livestock. Tsetse fly & Trypanosoma (T&T) constitute one of the greatest constraints on the African continent's socio-economic development limiting sustainable rural development and causing poverty and food insecurity. In 2001, the Organisation of African Unity (OAU), launched the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) to eradicate tsetse flies from Africa. The IAEA General Conference resolution GC (64)/RES/12/A.2 recognized the need to support the PATTEC to eradicate T&T by creating sustainable T&T free areas, using various suppression and eradication techniques such as the Sterile Insect Techniques (SIT).



Participants of the FAO/IAEA Coordination Meeting on Enhancing Regional Capacity for the Implementation of the Sterile Insect Technique as a Component for Area-Wide Tsetse and Trypanosomiasis Management 20–24 June 2022 Vienna, Austria.

In this scenario, the IAEA started a new regional project (RAF5087), which aims to provide technical support for the participating Member States to build and enhance their capacities for tsetse population control with the adoption of the SIT. The first regional coordination meeting of the RAF5087 project was attended by counterparts from 11 African Member States including Cameroon, Chad, Djibouti, Ethiopia, Kenya, Mali, Nigeria, Senegal, Uganda, United Republic of Tanzania, and Zimbabwe. The project counterparts fulfilled the objectives of the meeting by updating the progress achieved, lessons learned, and challenges faced at the national, and regional levels in the fight to control T&T.

During the meeting, technical discussions also focused on the Phase Conditional Approach to SIT application, since this is an essential tool during the preparation and implementation of SIT field projects. The meeting also discussed the support provided by the IAEA to its Member States through the previous regional project RAF5080, which included the provision of technical advice on different topics, reinforced during regional training courses, and implementation of fellowships and scientific visits. The meeting made recommendations on the planned activities focusing on the member state's current needs, technology impact, and resources availability. According to the

countries' priorities, three regional training courses will be organised in 2022–2023, focusing collecting entomological baseline data, genetic population studies and socio-economic assessments of T&T. At the end of the meeting, all participating Member States agreed with the outcomes and outputs of the current work plan, which were extensively discussed aiming to enhance regional capabilities, and harmonize procedures for the tsetse fly management at the regional level.

Taskforce Meeting, 15–18 November 2022, Vienna, Austria

In the last decade African Member States (MSs) have made many efforts to control the Tsetse flies, considered as the sole cyclical vectors of African trypanosomes. However, despite these efforts many challenges still exist. African Animal Trypanosomiasis (AAT) remains one of the greatest constraints on the African continent's socio-economic development limiting sustainable rural development and causing poverty and food insecurity.

The IAEA through its Technical Cooperation department and the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture organized a technical taskforce meeting to propose strategies and a roadmap to supporting the MSs in overcome challenges, bottlenecks, and key knowledge gaps on Tsetse & Trypanosoma (T&T) management at the regional level using sterile insect technique (SIT). This meeting also aimed to explore mechanisms to strengthen the African Union Pan African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC) activities since its role is relevant to support the MSs on the creation of sustainable tsetse and trypanosomiasis free areas in the region.



Participants of the Taskforce on Strengthening PATTEC Activities in Africa for the project RAF5087. 15–18 November 2022, Vienna, Austria.

The meeting had the participation of five external experts from Burkina Faso, Chad, Kenya, Senegal, and Zimbabwe. The invited experts are leading research and field activities on T&T management in their countries. The group agreed that inconsistent national funding, lack of national and regional policy, and inadequate commitment from local

governments, key stakeholders, and partners to support T&T management activities have been a common limitation.

During the meeting, technical discussions focused on successful programmes such as the operational projects implemented in Zanzibar (The United Republic of Tanzania) and Niayes region (Senegal) and the main challenges on T&T management as well as potential recommendations. Among the recommendations particular mention was made to the IAEA's support to revitalize the AU-PATTEC Coordination Office and to assist its MSs and AU-PATTEC on conducting feasibility studies following the Phased Conditional Approach (PCA) and Progressive Control Pathway (PCP) since both are fundamental tools for the planning, preparation and successful implementation of field projects aiming for T&T elimination. To summarize, the experts presented a final report requesting support from the IAEA through its TC mechanisms to continue enhancing MSs capacities in the region for their fight against the T&T problem.

FAO/IAEA Regional Training Course on Tsetse Baseline Data Collection and Data Management; 28 November–2 December 2022, Harare, Zimbabwe

The training was held at the Division of Veterinary Services and Department of Tsetse Control Services, Harare, Zimbabwe, and focused on technical aspects of field baseline data collection procedures, and data management. Both are key components for planning and implementing of an area-wide integrated pest management programme targeting tsetse flies. The participants included 33 professionals involved in field activities and data management from 16 Member States (Angola, Burkina Faso, Cameroun, Chad, Côte d'Ivoire, Djibouti, Ethiopia, Ghana, Kenya, Mali, Nigeria, Senegal, South Africa, United Republic of Tanzania, Zambia, and Zimbabwe).



Participants of the FAO/IAEA Regional training course on Tsetse Baseline Data Collection and Data Management. 28 November–2 December 2022, Harare, Zimbabwe.

The objectives of the training course were to: (1) enhance and develop knowledge, skills, and capability at the regional level for planning and implementing entomological baseline data surveys and (2) enhance and develop regional capabilities on database management systems for tsetse control programmes. In addition, the 'Tsetse and

Trypanosomosis Intervention Data Management System' was transferred for all participating Member States aiming to strengthened as well harmonized procedures on baseline data collection and management at the regional level. At the end of the training, the upcoming targets and priorities for the region related to these topics were also discussed and agreed on.

Enhancing Capacity for the Use of the Sterile Insect Technique as a Component of Mosquito Control Programs (RLA5083)

Regional Training Course on Handling, Shipment and Releasing procedures applied in SIT projects, 7–11 November 2022, Juazeiro, Bahia, Brazil

The training course was held at Moscamed Brasil from 7–11 November with the participation of 17 entomologists and technical staff from 14 member states of the Latin America and Caribbean Region including Antigua and Barbuda, Argentina, Bahamas, Brazil, Bolivia, Chile, Dominican Republic, Ecuador, Jamaica, Panama, Peru, Nicaragua, Saint Vincent and Grenadines, Uruguay, who are actively engaged in the project RLA5083, and participating in capacity building and SIT pilot trials in the region.



Participants of the IAEA Regional training course on Handling, Shipment and Releasing procedures applied in SIT projects. 7–11 November 2022 Juazeiro, Bahia, Brazil.

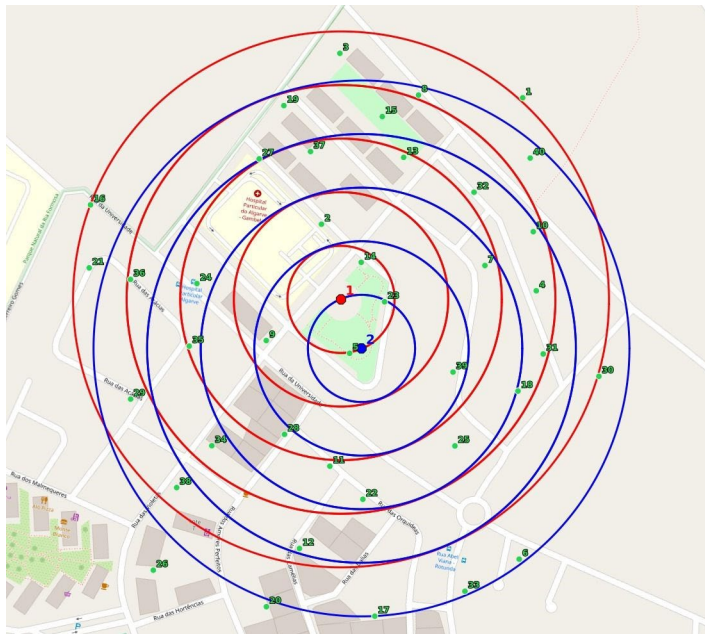
The training course was conducted by 5 international experts and focused on several aspects related to mosquito handling, quality control, shipping, and releasing procedures applied in SIT pilot trials targeting *Aedes* mosquito control as key components of the SIT package and included theoretical and practical sessions. The training also aimed to strengthen and harmonize procedures that are in common in SIT pilot trials at the regional level, including networking and information exchange. Along with the training, the participants attended several practical sessions that covered all steps of the workflow process in mosquito SIT project, from the mass-rearing to the sterile releases in the field. The participants also visited a pilot area and participated in a field release of sterile males of *Ae. aegypti*. Brazil, Mexico and the United States shared their challenges and lessons learned during the implementation of their SIT pilot trials. At the end of the training, a group dynamic

session was carried out in which the participants updated the country's status based on the Phase Conditional Approach (PCA), identified upcoming priorities, and discussed potential solutions to overcome challenges identified at the regional level for the successful implementation and validation of SIT field projects.

In conclusion, regional capacities and networking were strengthened and harmonized on the information about SIT package at the regional level.

Integrating the Sterile Insect Technique in the Control of the Invasive Vector Mosquito *Aedes albopictus* (POR5006).

Aedes albopictus, also known as Asian Tiger Mosquito, is considered as a major invasive species that has colonized to several European countries including Portugal. This species is also considered as an efficient vector of chikungunya, dengue and Zika viruses worldwide. *Ae. albopictus* has been associated with autochthonous transmission of some of the mentioned viruses in Europe since 2007. In Portugal, *Ae. albopictus* was reported for the first time in 2017 under the frame of the National Vector Surveillance Network—REVIVE (REde de VIGilância de VEctores), a programme coordinated by the Centre for Vectors and Infectious Diseases Research of the National Institute of Health (CEVDI/ INSA).



Map showing the configuration used to estimate the mosquito distribution in the target area from two single-release points (red and blue points). The green points represent the position and distribution of location of traps, concentric blue and red lines represent annuli which are 50 m from each other; Bottom: Human landing catch conducted during the study for sterile adult collections.

Since its finding, the seasonal activity of *Ae. albopictus* has been reported annually by the REVIVE in the two affected areas in Portugal, one located in the North of Portugal, and the other in southern region of Algarve.

In the absence of an efficient control strategy, this invasive mosquito species will easily disseminate and establish in other Portuguese regions, and thus increasing the risk of mosquito-borne disease outbreaks. In this context, and to mitigate the potential impact of *Ae. albopictus* in transmitting human diseases in the country, Portugal is willing to evaluate and validate the SIT technology as a sustainable tool to suppress *Ae. albopictus* populations at the national level. For this, Portugal has undertaken an SIT pilot project in Gambelas, a neighbourhood located in Faro municipality, southern region of Algarve. The project is being implemented by the CEVDI/ INSA with the technical support of the IAEA. In October 2022, the first Mark, Release Recapture (MRR) trial was conducted.



Mark, release recapture (MRR) study in Faro, Portugal.

The local staff received a training on Geographic Information System (GIS) tool and spatial data management prior to the trial. Around 100 000 sterile mosquitoes in total were produced at the Centro Agricultura Ambiente 'G.Nicoli' Italy and shipped to Portugal three times on a weekly bases from 10–25 October, which required the implementation and optimization of a protocol for packaging and transboundary shipping chilled sterile males over a long distance.

Based on the gathered information from this first trial, the mean and maximum distance travelled by the released sterile males from the release point was estimated at 185 and 297 meters, respectively. Sterile males were caught until six days after the first release, even though the greatest number of recaptured sterile males (98%) was observed within the first three days after the first release. These results, together with knowledge about wild population density and its spatial distribution, will be crucial for developing the release strategy, including the release frequency and planning the intervention phase aiming at the suppression of *Ae. albopictus* in the target area. This first trial was also essential to develop and optimize local procedures as well as to train the local team for the implementation of sterile mosquito releases in open field conditions in the future.

Enhancing the Capacity and the Utilization of the Sterile Insect Technique for *Aedes* Mosquito Control (RAS5095)

FAO/IAEA Regional Training Course on Methods for the Mass-Rearing, Irradiation and Release of Sterile Male *Aedes* Species. 21–25 November 2022, Singapore

The regional training course was organized by the National Environment Agency (NEA) of Singapore and attended by 19 international participants from 11 Member States (Bangladesh, Indonesia, Iran, Jordan, Malaysia, Mongolia, Pakistan, Philippines, Sri Lanka, Thailand, Viet Nam). During the training course, the participants shared their experiences and perspectives, and learned about the ways to develop the sterile insect technique (SIT) for *Aedes* mosquito control in their respective countries.



Participants of the Regional Training Course on Methods for the Mass-Rearing, Irradiation and Release of Sterile Male *Aedes* Species, 21–25 November 2022, Singapore.

They had the opportunity to visit NEA's Environmental Health Institute facility which is currently producing 5 million sterile male *Aedes aegypti* weekly with cutting edge automatized mass-rearing technologies. Their efforts have led to successful reduction of the dengue vector mosquito population by up to 98% at areas with at least 1 year of releases of irradiated male *Wolbachia-Aedes* mosquitoes, and a reduction of dengue cases by up to 70% during this year's huge dengue outbreak in Singapore.

The training included an overview of SIT and incompatible insect technique (IIT)-SIT field trials at the world level, with emphasis on the phased conditional approach to test mosquito SIT and specific presentations on some advanced projects like China, Singapore and Spain. It included lectures on mass-rearing, handling, irradiation, and release of sterile males. Also, it included practical exercises on

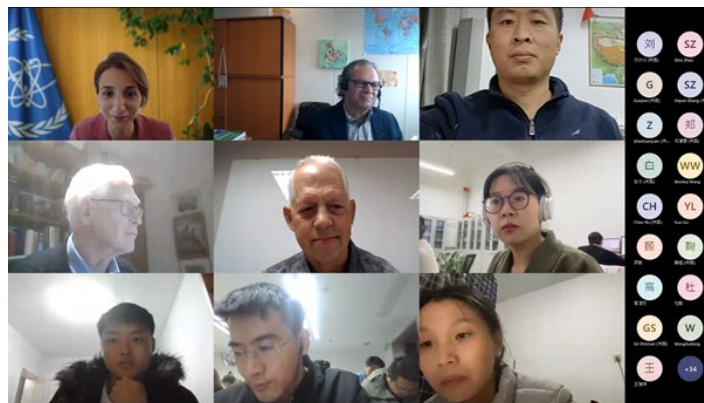
irradiation, quality control, data management and marking of the sterile males, as well as a field visit to a target area with participation to the release of sterile males.

Lessons learned from the training will be instrumental in helping Member States who have successfully conducted small-scale field trials under regional TC projects in Asia and the Pacific – namely Indonesia, Malaysia, Sri Lanka and Thailand—to upscale SIT for the next phase of activities.

Demonstrating Feasibility of the Sterile Insect Technique in the Control of the Codling Moth, *Cydia pomonella* (CPR5027)

FAO/IAEA Training Course on Codling Moth Area-wide SIT Implementation, 10–19 October 2022, virtual

A virtual training course was carried out from 10–19 October 2022 with the participation of 54 fellows from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS), Shenyang Agricultural University, Henan Institute of Science and Technology, and Guizhou Academy of Tobacco Science.



Virtual training course on Codling moth area-wide SIT implementation.

A total of 20 hours of lectures were offered including a number of videos to illustrate the mass-rearing and field operation processes for lepidoptera. The topics covered were introduction to area-wide integrated pest management and the sterile insect technique, mass-rearing and sterilization, sterile moth release, quality control, design of rearing facilities, facility management, packing moth for long-distance shipping, ground, aerial and drone releases, costs and benefits associated to programme operations.

This event is part of the activities aimed at capacity building for future application of area-wide sterile insect technique (SIT) against the codling moth in China.

Coordinated Research Projects (CRPs)

Project Number	Ongoing CRPs	Project Officer
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
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)	Jeremy Bouyer
D4.10.29	Improving Rearing, Handling, and Field Components for Fruit Fly SIT Application (2021–2026)	Walther Enkerlin
D4.10.28	Improve the Mass-Rearing of Lepidoptera Pests for SIT Programmes (2022–2027)	Daguang Lu

Second RCM of the CRP on Mosquito Irradiation, Sterilization and Quality Control. 18–22 July 2022, Vienna, Austria

Sixteen scientists from 15 countries travelled to IAEA Headquarters to present their results achieved at the past year, and two additional participants presented virtually. The results have been achieved so far are highlighted below:

First, several teams reported results confirming their suitability of X ray for sterilization of mosquitoes for SIT pilot trials.

Second, preliminary studies using a 10MeV industrial e-beam device has shown to successfully irradiate up to 500 million fruit flies per week and has shown promising results and application potential for mosquito sterilization. E-beams might thus represent a suitable alternative to panoramic irradiators in the future.

Third, following the series of experiments to identify factors in mosquito irradiation that may affect dose-response in terms of sterility, additional variables have been investigated to further standardize irradiation procedures such as pupae density dependent hypoxia. Also was found that adult irradiation, adult age and the geographic origin of mosquito strains seem to have little or no effect in the studies performed thus far.

Fourth, In SIT/IIT strategy, the impact of irradiation on *Wolbachia* densities is an important topic. Recent studies have found that doses up to 45Gy did not reduce *Wolbachia* densities in *Aedes albopictus*.



Participants of the Workshop on Irradiation methods and dosimetry at the Insect Pest Control Laboratory, 14–15 July 2022, Seibersdorf, Austria.

Combined with this RCM, a workshop on Irradiation methods and dosimetry was conducted at the Insect Pest Control Laboratories (IPCL) on 14–15 July 2022. Ten scientists from the RCM participated and presented their current irradiation and dosimetry protocols, and discussed

the issues and queries within the group. Presentations on irradiation and dosimetry basics, including how to design an irradiation set-up according to the experiments, how to obtain a dose response curve, how to irradiate adults vs pupae, and the importance of dosimetry and for calibration and use of Gafchromic films were accompanied by practical group sessions. Through the workshop, the participants were familiarized with the gamma irradiators Foss Model 812, Nordion Gammacell220, and X-ray irradiators Best Theratronics Raycell MK2, and Radsourc RS2400. The main differences between the devices regarding dose rates and chamber geometry and capacity were discussed, and participants were shown how to use dosimetric films to measure absorbed dose, to read the film absorbance and to create a canister dose map.

First RCM on Improve the Mass-Rearing of Lepidoptera Pests for SIT Programmes. 5–9 September 2022, Vienna, Austria

The RCM was attended by 23 participants from 14 countries including Argentina, Australia, Canada, Chile, China, Indonesia, Iran, Malaysia, Mauritius, Pakistan, South Africa, Syrian Arab Republic, United States of America and Viet Nam.

During the first two days of the meeting, the CRP agreement and contact holders presented their current research relevant to the CRP, and the research plans for the first year of the CRP. During the last three days of the meeting, general discussions were held to define and review the thematic areas of the CRP, the general and specific R&D objectives to be addressed during the entire five years of the CRP, and the CRP Logical Framework, in order to agree on minimum outputs to be achieved at the end of the CRP. Furthermore, participants were divided into two working groups to develop more detailed R&D plans to be conducted during the first 18 months of the CRP.



First RCM on Improve the Mass-Rearing of Lepidoptera Pests for SIT Programmes. 5–9 September 2022, Vienna, Austria.

Mr Des Conlong from South Africa was invited to have a presentation on ‘The Comparative Slaughter Technique for the Insect Diet Development’. The participants discussed the possibility to use this technique on the development of insect diet. It was agreed that the workshop on carcass milling

technique that was proposed in conjunction with the second RCM will be replaced by a diet and host chemical analyses organized by IAEA. All participants identified three main hosts (including the plant part(s) and crop stage) of their working species. The FAO/IAEA will identify a laboratory/company for Proximate and Amino Acid Analyses.

The overall objective of this new CRP approved for the period 2022–2027 is to expand and improve the sterile insect technique for use against lepidopteran pests of crops to improve food security and enhance health and well-being. It focuses on three areas of mass-rearing for Lepidoptera SIT: (1) develop basic rearing for species that have potential for SIT but with no artificial rearing established, (2) scale-up rearing technology for species where rearing is a low scale and (3) improve mass rearing for species under operational programmes.

Third RCM on Assessment of Simultaneous Application of SIT and MAT to Enhance *Bactrocera* Fruit Fly Management. 20–24 November 2022, Sydney, Australia

The RCM was hosted by Macquarie University, Australia and attended by 29 research contract and agreement holders, as well as observers from 19 Member States including Australia, Austria, Bangladesh, Brazil, China, Czechia, France, India, Israel, Japan, Kenya, Malaysia, Mauritius, New Zealand, Pakistan, South Africa, Thailand, United States of America, and Viet Nam.



*Participants of the third RCM of the CRP on Assessment of Simultaneous Application of SIT and MAT to Enhance *Bactrocera* Fruit Fly Management. 20–24 November 2022, Sydney, Australia.*

The progress achieved during the last 18 months and the individual workplan in the next 18 months were reported. It covered all the research themes of the CRP, such as (1) pre-release treatment methodology for reduced lure response; (2) effects of pre-release treatments on fly performance; (3) novel attractants - microbiota, cuticular lipids, and plant extracts; (4) selection for non-responders; (5) mechanisms of lure response and (6) semi-field and field assessments of

SIT + MAT simultaneous application. In addition, two presentations were delivered by the observers from Japan and the United States of America.

The logical framework (LFM), five years workplan, next 18 months individual workplan and meeting report were reviewed and discussed during the following days of meeting. The proposal for the special issue in the end of CRP was also discussed.

Final RCM on Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management. 08–12 November, Sydney, Australia

The RCM was attended by 12 research contract and agreement holders, as well as observers from Argentina, Brazil, Chile, Egypt, France, India, Italy, and New Zealand.



Participants of the fourth and final RCM on Integration of the SIT with Biocontrol for Greenhouse Insect Pest Management at the Macquarie University, Sydney, Australia.

Fifteen presentations on the progress of their research were delivered, and the quality was of high standards. The various topics in the presentations covered all expected outputs of the CRP, focus on the advance development and implementation of SIT for integration with other biocontrol in greenhouses.

The following are some of the main achievements of this CRP:

- A dose response for *Drosophila suzukii* was established and 200Gy selected as an optimal sterilization dose for SIT.
- Mating behaviour trials for *D. suzukii* were performed between sterile male and wild fertile females to establish competitiveness and compatibility between different populations. No barriers on sexual compatibility to irradiated sterile male of *D. suzukii* were observed.

- In field cage experiments, effectiveness of sterile male insect release by using different overflooding ratios showed population suppression. As few as 40:1 (sterile:wild) male *D. suzukii* reduced the number of offspring produced.
- Biological control by means of natural enemies insect, entomopathogenic bacteria and fungi was assessed for efficacy in managing *D. suzukii* populations alone and in combination with SIT.
- The estimation of *D. suzukii* population size based on trap efficacy and numbers of flies caught were developed to inform numbers of sterile flies needed to overflood the wild fly population by use of population models.
- Informative molecular markers of *D. suzukii* were identified and validated for the analysis of mating behaviour.
- Guidelines for mass rearing and quality control protocols for *D. suzukii* have been published and are available online <https://www.iaea.org/sites/default/files/massrearing-and-irradiation-swd.pdf>.
- Dose response for the target Lepidoptera species *Tuta absoluta* has been established.
- The effects of sub sterilizing doses on parental and F1 performance parameters in *Helicoverpa armigera* and *Spodoptera littoralis* has been established.
- Mating behaviour trials have been performed between sterile males and fertile females and competitiveness trials have been completed for *T. absoluta*, *H. armigera* and *S. littoralis*.
- Different diets for *T. absoluta*, *H. armigera* and *S. litura* have been assessed and the candidate local available ingredients for mass rearing were identified and optimized.
- No asexually reproducing populations of *T. absoluta* have been detected in Argentina.
- The presence of *Wolbachia* in *T. absoluta* has been surveyed in India and Argentina and genotyping using MLST and wsp analysis has shown that the *Wolbachia* strain from India are of Supergroup A and B and *Wolbachia* strains from Argentina are of Supergroup B.
- The parasitization efficiency of different biocontrol agents like predator, parasitoids and fungi has been evaluated for the control of *T. absoluta*, *H. armigera* and *S. littoralis*.

These results will help to facilitate the implementation of SIT for the target species of the CRP.

Developments at the Insect Pest Control Laboratory (IPCL)

Genetics and Molecular Biology

Establishment and Quality Control Analysis of An *Aedes aegypti* Red-eye Genetic Sexing Strain with Pakistani Genomic Background

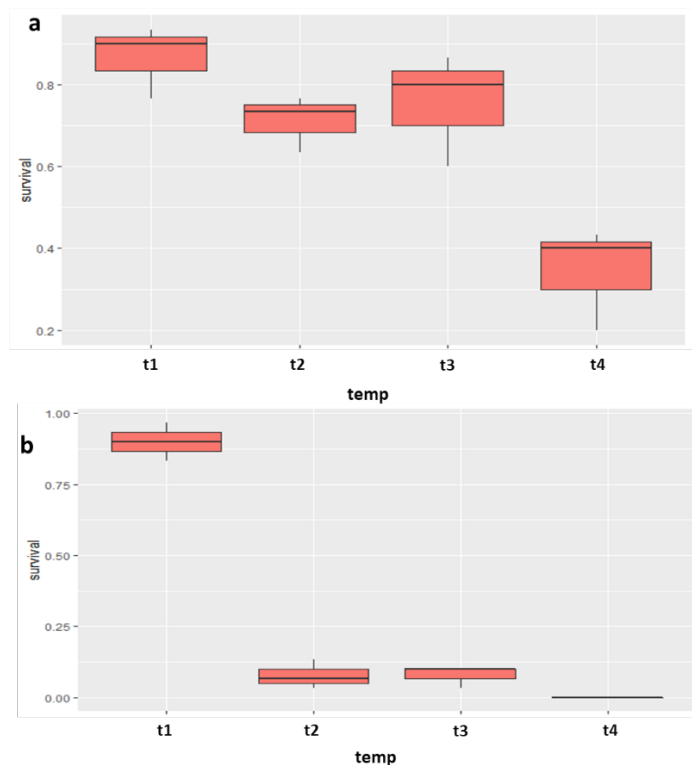
We have recently reported on the development of an *Aedes aegypti* genetic sexing strain (GSS) using classical genetic approaches and a visible trait, eye colour, as a selectable marker. In this GSS, namely Red-eye GSS, males have black-eyes and females have red-eyes. We also induced a chromosomal inversion (Inv35) covering the region of the red-eye locus to increase its genetic stability and minimize the number of recombinants, that is, red-eyed males and black-eyed females. In addition, we introgressed both the genetic sexing trait of the Red-eye GSS and the inversion Inv35 into six different genetic backgrounds, i.e., Brazil, Indonesia, Mexico, Singapore, Sri Lanka, and Thailand, and we showed that the new strains maintain their sexing properties and present enhanced genetic stability.

We have expanded the previous studies by introgressing the red-eye mutation and the inversion into a Pakistani genetic background thus producing and evaluating two new strains, namely Red-eye GSS-PAK and Red-eye GSS/Inv35-PAK. Through a series of experiments, their genetic stability and biological quality were studied before they could be considered for any small- or large-scale sterile insect technique (SIT) field application. By monitoring the two strains over several generations, we observed a small number of recombinants between 1-3% in the Red-eye GSS-PAK, which was greatly reduced to 0.1-0.6% in the strain carrying the chromosomal inversion, Red-eye GSS/Inv35-PAK. This means that once a sex sorter is developed based on this selectable marker, the female contamination could be kept at minimal levels well below the recommended threshold of 1%.

We also evaluated the biological quality of the two GSS along with the wild-type strain which was used for the introgression experiments. The results clearly showed that the introgression can have a positive or a negative impact and this depends on the strain and fitness trait studied. Positive effects were observed in respect to the fecundity (both GSS), and the flight ability of males (Red-eye GSS/Inv35-PAK), while negative effects were recorded in fertility (Red-eye GSS/Inv35-PAK), pupal and adult recovery (Red-eye GSS/Inv35-PAK), and survival rate of males (both GSS). On the other hand, there was no impact on the pupation rate or the pupal weight of males and females, and the survival rate of females in the two GSS.

The use of local populations has been recommended for small- or large-scale sterile insect technique applications against populations of mosquito species such as *Aedes aegypti* and *Aedes albopictus* as they are major vectors of human pathogens such as dengue, chikungunya, Zika, and yellow fever. Therefore, the introgression process is necessary if it is to use the genetic sexing characters (and / or chromosomal inversions) originally developed in a different genetic background. Alternatively, a genetic sexing strain should be developed *de novo* using a colony established from the target local population. In either case, the genetic stability, and the biological quality of the GSS, including its productivity, flight ability, longevity and male mating competitiveness, should be thoroughly assessed before its use in small- or large-scale field applications.

Aedes aegypti Thermal Treatments



Thermal exposure results of an *Aedes aegypti* (a) temperature-resistant, and (b) temperature-sensitive strain.

Conditionally lethal selectable markers are in high demand for the construction of genetic sexing strains (GSS), as they allow for selection of males and removal of females from the rearing process. The earlier in development the lethal marker is expressed, the more beneficial. It is considered for mass-rearing facilities and large-scale operational programs in terms of cost and labour. Temperature sensitive lethal (*tsl*) traits are the most sought-after markers, especially after the impeccable record of the *tsl* gene in *Ceratitis capitata*. In

Aedes aegypti we are searching for temperature resistant and temperature sensitive phenotypes that could be utilized for the construction of a GSS that would comprise a *tsl* phenotypic marker. Towards this effort, IPCL has conducted diligent research and developed a thorough protocol for exposing *Ae. aegypti* to thermal treatments. During the protocol development, the strain variability, the genetic background of the strains, exposure temperatures, durations and devices were taken into account, while several technical parameters were scrutinized to reassure the formation of a uniform approach.

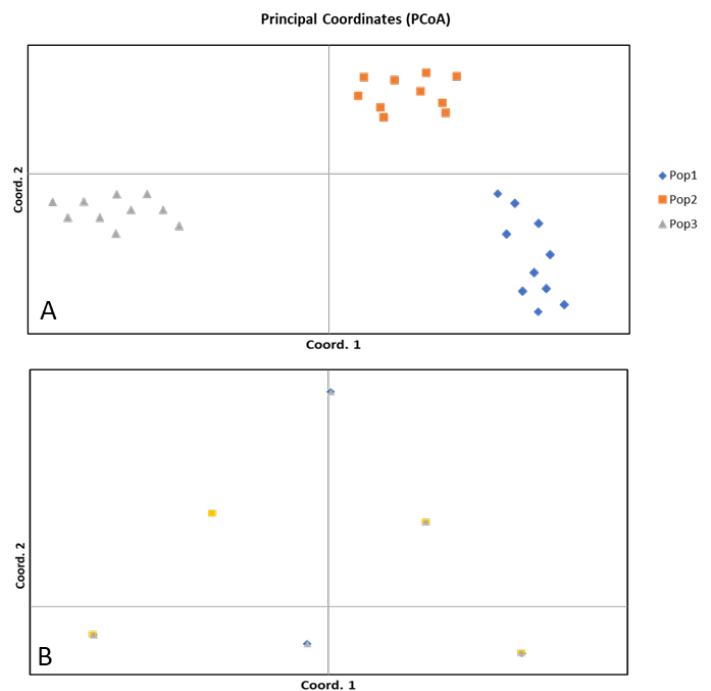
Thermal exposure of *Ae. aegypti* revealed several temperature-resistant and -sensitive strains while heterogenous results that were declaring lack of phenotypic homogeneity, were also present. Strains with a more pronounced thermal pattern were subsequently analyzed genetically through single pair crosses. The results of these crosses can reveal whether the resistance or sensitivity expressed by specific strains is a monogenic or polygenic trait and can drive further research for the construction of a *tsl*-based *Ae. aegypti* GSS.

Microsatellites: A Powerful Molecular Tool for Genetic Analysis in Support of Sterile Insect Technique

Microsatellites are short tandem DNA repeated units, usually between two to six base pairs in length. They can be found dispersed in coding and non-coding regions in all prokaryotic and eukaryotic genomes. The number of repeats may differ between individuals in different populations. As microsatellites present high mutation rates and generally have high number of alleles per locus, they can unravel high levels of heterozygosity. Due to their abundance in the genome, high degree of polymorphism, and easiness of isolation, they constitute a very powerful tool, which has been used extensively in genetic fingerprinting, paternity testing, genetic linkage analysis to identify a gene or a mutation responsible for a given phenotype as well as in population genetic analysis to assess the relationships between different populations and individuals.

Microsatellite analysis is also very useful in entomology including SIT-related studies such as the monitoring of genetic changes which may be occurring during laboratory domestication of an insect population. Several studies have reported that life traits can be affected during the laboratory adaptation process, including fecundity, fertility, developmental time, lifespan, dispersal ability and stress resistance. Any decrease in the quality of desirable biological traits may have a significant impact on small- or large-scale SIT applications against insect pests and disease vectors. The loss of genetic diversity might be a rapid process for some species taking place quite early after the introduction of the wild population in the laboratory. Therefore, it is essential to monitor the overall genetic diversity and the genetic changes which may be occurring during mass-rearing as these may

affect the biological quality of a mass-reared strain including its male mating competitiveness. If significant changes are observed, action steps need to be taken by enriching mass-reared colonies with fresh insect material introduced from the wild.



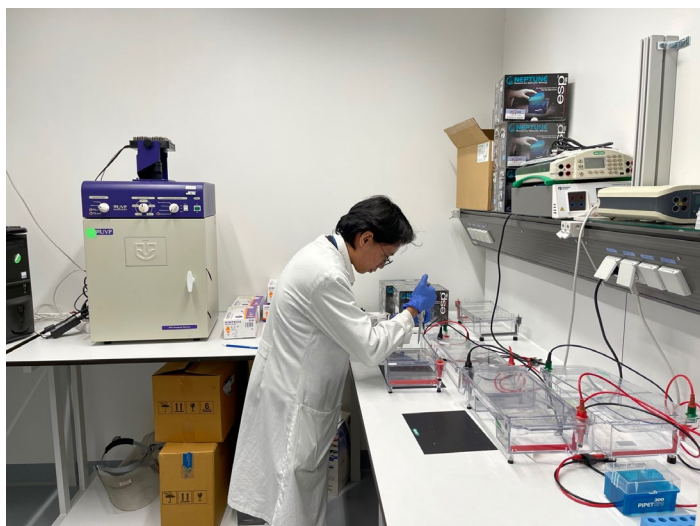
Principal coordinate analysis (PCoA) analysis of three different insect populations (Pop1, Pop2, Pop3) using Nei's genetic distance matrix. Each population consisted of 10 insect individuals. (top) The results of a high polymorphic marker A are presented. Three populations are distinguished as three different clusters. (bottom) The results of a low polymorphic marker B are presented. The dots representing the samples overlap (multicoloured dots).

There are ongoing studies at the IPCL to monitor genetic changes during the laboratory domestication and rearing of populations of SIT targeted insect pests and disease vectors. An essential step during microsatellite analysis is the selection of markers. The most 'informative' subset of microsatellite markers should be selected in each analysis. The figure presents a principal coordinate analysis (PCoA) using three insect populations. The samples were analyzed with two different microsatellite markers, a high (marker A) and a low (marker B) polymorphic marker. In the top graph of the figure, marker A can distinguish the three different populations. This is not the case in bottom graph of the figure with the marker B, where the dots representing the samples overlap, making it difficult to distinguish the three populations.

Plant Pests

Development of Microsatellite Markers for Population Genetics Studies of the Cocoa Pod Borer *Conopomorpha cramerella*

The cocoa pod borer *Conopomorpha cramerella* (Snellen) (CPB) is one of the most devastating insect pests of cocoa throughout South-East Asia. The species is present in the Philippines, Malaysia, Indonesia and Papua New Guinea. Although, there are various control measures that have been implemented to manage populations of this pest, the SIT is being considered as an additional control tactic for integration in area-wide integrated pest management (AW-IPM) approaches. There is little or no information available on the population structure of this pest in the infested countries and population genetics studies could help answer the important question whether it is the same population that is infesting these countries or not. This information is important for SIT programmes as it will allow an assessment on the degree of isolation of these different populations in these different countries.



Ms Marynold Purificacion preparing the cocoa pod borer (CPB) samples.

To this end, developing tools such as microsatellites to analyse the genetic structure of the CPB populations is required. Staff of the IPCL have developed 12 microsatellites for CPB and their efficiency to explore the genetic diversity between and within populations is being assessed. The quality control studies of these microsatellites proved that they are sufficiently polymorphic to explore the genetic differences of the different CPB populations. Moreover, the microsatellites developed for the CPB, were tested against the litchi stem end borer *Conopomorpha sinensis* (LSB) collected in Viet Nam. The results indicate that 9 microsatellites can be used to analyse the population genetics of this species. This work was carried out by Ms Marynold Purificacion, a TC fellow (under TC project RAS5086) from the Philippines.

Mating Compatibility and Competitiveness Studies of the Black Pupae Genetic Sexing Strain AF-IPCL-89 of *Anastrepha fraterculus* with Wild Populations of Argentina and Peru

The Plant Pest group of the IPCL developed a genetic sexing strain of the South American fruit fly *Anastrepha fraterculus* (AF-IPCL-89) based on a colour mutation of the pupae (black females–brown males), which will allow separation of the sexes at the pupal stage. The next step is to evaluate the genetic stability of the strain and its production and quality control profile under mass-rearing conditions and to assess its performance under field conditions. It is known that the nominal species *A. fraterculus* is a cryptic species complex and previous studies have demonstrated the existence of high levels of mating isolation between populations from Argentina and the coastal areas of Peru. Further studies have also shown high levels of pre-zygotic or reproductive isolation as the hybridization of these populations resulted in reduced egg viability and sex distortion of the progeny. These data have clearly shown that these two populations belong to different biological entities within the *A. fraterculus* cryptic complex. The reproductive isolation of the different populations has serious implications for SIT programmes, as colonies that would produce the sterile males for different countries need to be derived from the target population in each country.

Therefore, before transferring the black pupae GSS to Member States, it is mandatory to assess its mating compatibility with populations from the target areas of each respective country.

Argentina – Mr Diego Segura from Argentina, was hosted at the IPCL for a 3-month fellowship supported by TC project RLA5087. He carried out a series of field cage tests to assess the mating competitiveness and compatibility of males from the *A. fraterculus* black pupae GSS. The males from the black pupae strain were tested against males from four populations from Argentina: CABA, Concordia, Tucuman, and Yuto. Males of the GSS and the wild-type strain were released together with virgin wild females in large walk-in cages deployed in the ecosphere of the IPCL. Results from 8 replications of the test with each population showed no differences in mating success between males from each of the tested populations and those from the AF-IPCL-89. These positive results support the import of the AF-IPCL-89 to Argentina for its future use in SIT programmes against this pest in Argentina.

Peru – Mr Edgardo Lopez Ortiz from Peru was hosted at the IPCL for a 6-month fellowship, supported by TC project RLA5087. The aim of his fellowship was to carry out backcrosses and crosses between wild males from Peru and black pupae females from the GSS AF-IPCL-89, as well as translocated males from AF-IPCL-89 and wild females from Peru. The GSS AF-IPCL-89 was originally derived from wild flies collected in the south of Brazil and the north of Argentina. To overcome the sexual incompatibility between

the Peruvian and GSS AF-IPCL-89 flies, a series of introgressions ($n = 6$) will be carried out with flies that have a genetic background from Peru into the GSS AF-IPCL-89. That will allow us to use the same male translocation and the black pupae phenotypic marker to create a new GSS from Peru. Results from earlier studies have already reported the significant sexual incompatibility between these two populations.



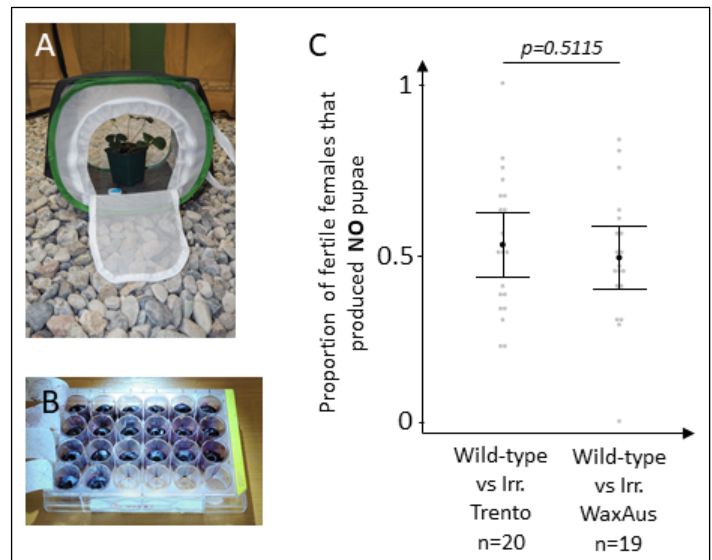
Mr Edgardo Lopez Ortiz separating mature *Anastrepha fraterculus* male and female adults for mating compatibility tests.

After three introgressions, Mr. Ortiz intends to conduct a mating compatibility test to determine whether the sexual incompatibility is ceding. A final assessment will be done after completing the total planned introgressions.

Validation of a methodology for the evaluation of male competitiveness in *Drosophila suzukii*

A methodology for assessing the competitiveness of mass-reared *Drosophila suzukii* males was described in 2021 in collaboration with researchers from the French National Research Institute for Agriculture, Food and Environment (INRAE). This methodology was successfully validated in autumn 2022 after some minor adjustments.

Briefly, sterile mass-reared and fertile wild-type adults of both sexes are left to interact for a day under ecologically realistic conditions. The females are then caught and held individually for an additional day in wells that contain a half fruit placed on larval diet. Females are then identified as fertile or sterile due to their marking with fluorescent dust and removed. Ten days later, the presence of pupae in the wells that contained fertile females makes it possible to deduce the proportion of fertile females that have mated with fertile wild-type males. The validation of the method allowed us to compare the biological quality of the males of two different IPCL mass-reared lines of *D. suzukii*. The Trento line, established in 2014, and the new WaxAus line, obtained from a series of crosses between the Trento line and a wild-type line, showed similar levels of competitiveness.

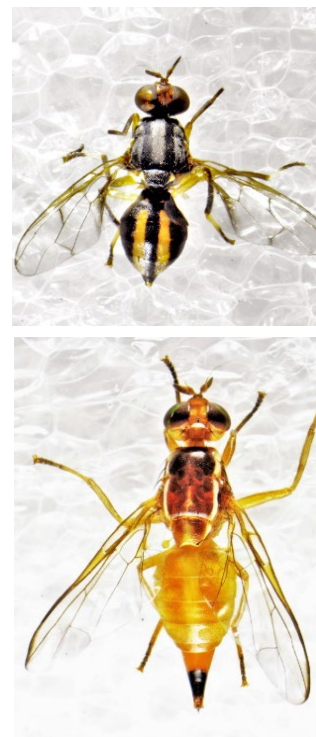


Model of a cage used for the protocol (A). Wells (containing half fruits and larval diet) used to maintain for a day females captured from a single cage (B). Estimates of the competitiveness of *Drosophila suzukii* mass-reared males (Trento and WaxAus lines). Observed mean (black dot), CI 95 % (bars), raw data (grey dots). Comparison between the two cages: linear mixed model, $\alpha=0.05$ (C).

The very details of the methodology will be published soon and will provide partners with a reproducible Quality Control protocol adapted for *D. suzukii*.

Bactrocera kirki and *Bactrocera xanthodes*

Mr Panapansa Koroï Rakuita from Fiji, supported by national TC project FIJ5003, has been working on the colonization of *Bactrocera kirki* and *Bactrocera xanthodes* during his 6-month TC fellowship. These fruit fly species are important economic pests, hampering fruit production and trade in the Polynesian Islands.



Bactrocera kirki (top) and *Bactrocera xanthodes* (bottom).

Mr Panapansa has managed to establish colonies of both species at the IPCL by rearing them on mangos. A first successful attempt was made to rear both fruit flies on an artificial diet, indicating that both populations were already adapted to laboratory conditions. During the last part of his fellowship, Mr Panapansa is focussing on fine-tuning the rearing protocols for both species. The establishment of these two colonies using artificial rearing protocols will allow the use of the SIT to manage populations of these two species in infested islands of Polynesia.



Mr. Panapansa Koroi Rakuita checking the viability of eggs of *Bactrocera kirki* collected in artificial eggging devices.

FAO/IAEA/USDA Phytosanitary Treatment Project



Ms Inajara Viana Gomes presenting research on phytosanitary irradiation for *Drosophila suzukii* at ICE 2022.

A small number of adults emerged from irradiated pupae reared on blueberries in the first phase of the confirmatory tests conducted to validate a phytosanitary irradiation dose for *Drosophila suzukii*. As a result, the second phase of confirmatory tests using *D. suzukii* reared on cherries was initiated, aiming to achieve a minimum number of 30 000 emerged adults from the irradiated pupae. The results from both experimental phases suggest that a dose of 80 Gy prevents development of the F₁ generation in *D. suzukii*

reared on blueberries and cherries. The preliminary findings on phytosanitary irradiation against *D. suzukii* were presented at the XXVI International Congress of Entomology by Ms Inajara Viana Gomes, a Ph.D. student at the IPCL.

Livestock Pests

Effect of Long-term Mass-rearing on the Genetic Diversity of *Glossina palpalis gambiensis*

Tsetse flies are the vectors of sleeping sickness and nagana in sub-Saharan Africa. The fight against these diseases is mainly based on vector control in the absence of effective vaccines. The sterile insect technique (SIT) in the frame of an area-wide integrated pest management (AW-IPM) approach was successfully used to eradicate a *Glossina austeni* population on Unguja Island of Zanzibar, a *Glossina palpalis palpalis* population in Nigeria and *Glossina palpalis gambiensis* and *Glossina tachinoides* populations from an agro-pastoral area of Sidéradougou in Burkina Faso.

There is very little information available on the genetic stability of tsetse fly colonies, especially when they have been maintained in the laboratory for long periods. Introducing wild flies periodically to refresh the colony is very difficult as it requires adaptation of the wild flies to the *in vitro* feeding system. As a result, tsetse colonies are maintained for many years without any measurement on its genetic diversity which might affect the males' performance and the compatibility of the produced sterile males with wild virgin females.



Mr Mikhailou Dera preparing the *Glossina palpalis gambiensis* samples.

We addressed this question and carried out a genetic characterisation of different colonies of *Glossina palpalis gambiensis* that had been colonized for different time periods. This included the Centre International de Recherche en Afrique (CIRDES) colony which has been maintained for more than 40 years and other colonies that have been maintained for shorter periods (The Insectarium

de Bobo Dioulasso (IBD) colony, The Senegal colony (SEN) in addition to two hybrid colonies: (i) an introgression of the SEN colony and the CIRDES colony named ICIRSEN and (ii) a cross between CIRDES and IBD colony named HCIRIBD). The study was carried out using microsatellites and the results indicate two clusters of flies, one cluster containing SEN and ICIRSEN and a second cluster with CIRDES, IBD and HCIRIBD. There was no indication of significant differences between the CIRDES and IBD colony. This work was conducted by Mr Mikhailou Dera, a consultant from Burkina Faso.

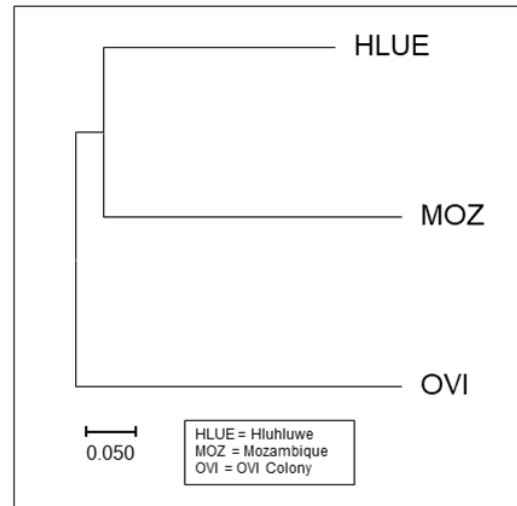
Development of Microsatellite Markers for *Glossina brevipalpis* Population Genetics Studies

The SIT is species-specific and therefore, the mass-reared and released sterile males should be compatible for mating with the target species in the field. Details 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 important 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.

There were no microsatellite markers available for *Glossina brevipalpis*. The development of microsatellite markers for this species was conducted at the IPCL by Mr Fabian Gstöttenmayer, a PhD consultant from Austria. The results indicate the availability of 8 primers that can be used as microsatellite markers for *G. brevipalpis*. The quality control of these microsatellites indicated their suitability for population genetics studies. The microsatellites were able to distinguish between the genetic structure of *G. brevipalpis* collected from South Africa, Mozambique and the colony flies originated from Kenya.



Phylogenetic tree of *Glossina brevipalpis* populations.

Radiation Sterilization of *Glossina fuscipes fuscipes* Pupae

The sterile insect technique for tsetse flies relies on the release of competitive sterile males, however many factors can have a negative effect on male competitiveness, and this is especially so when these males are produced far away from their release sites. Many of these factors have been highlighted during the tsetse eradication programme in the Niayes region of Senegal. Male tsetse pupae were produced in Burkina Faso and Slovakia, sterilized with X- or γ -radiation and shipped biweekly to Senegal. This entailed handling of the pupae numerous times, sterilizing them at different pupal developmental ages and shipping them long distances. These current handling, radiation and shipment protocols were customized for the Senegal situation to mitigate as much as possible the effects of these factors on the competitiveness of the sterile males.



Mr Mahamat Hissene (left) and Mr Aristide Kabore (right) assessing reproductive status through dissection of *Glossina fuscipes fuscipes* females that were sterilised or that were mated with sterilized males.

An SIT program has also been proposed for the Mandoul human sleeping sickness focus in Chad. Similar to the SIT program of the Niayes region of Senegal, the sterile males will be produced outside of Chad and shipped long distance following similar protocols. However, it is recommended that customization of these protocols needs to be done and to that end, Mr Mahamat Hissene, a fellow from Chad, was hosted at the IPCL to assess the effect of gamma radiation on female and male *Glossina fuscipes fuscipes* pupae of different ages. Twenty-six- and twenty-nine-day old pupae of both sexes were exposed to γ -radiation in air with doses ranging from 80 to 140Gy. A reduction in emergence rate was observed for 26-day-old pupae when exposed to a radiation dose of 120Gy and above, however this reduction was not seen for 29-day old pupae. On average female flies were more sensitive to radiation as a dose of 80Gy inhibited all production of pupae. For the males a dose of 80Gy was sufficient to induce 95% and 99% sterility in females that mated with males that were exposed as pupae on day 26 and 29 post-larviposition, respectively. Radiation dose or pupal age did not affect the male's ability to transfer sperm to the female. For both females and males, higher mortality rates were observed for higher radiation doses and when pupae were exposed at younger ages. The radiation protocols for *Glossina fuscipes fuscipes* pupae can now be updated. Further quality control assessments on flight propensity and male mating competitiveness are planned to follow.

Human Disease Vectors

Effect of Rearing Temperature on Larval Development and Pupal Production of *Aedes* mosquitoes

Abiotic factors such as temperature and relative humidity are known to affect mosquito's life-history traits. However, available studies aiming at understanding the effect of temperature focussed on large temperature variations (for example 5-degree shift). It is not well understood whether a slight temperature variation can affect mosquito larval development, synchronization, pupal yield and adult fitness. Recent observations showed significant variations in pupal yield under the recommended rearing temperature of $28\pm 2^{\circ}\text{C}$, which is used for rearing *Aedes* mosquitoes at the IPCL and other laboratories. Therefore, experiments are being conducted to examine i) whether a slight temperature variation (single degree temperature shift) can affect mosquito larval development, synchronization, pupal yield and ii) whether both *Aedes albopictus* and *Aedes aegypti* can be reared under the same temperature conditions. *Aedes aegypti* and *Ae. albopictus* were reared simultaneously inside climate-controlled chambers simulating the conditions used in mass-rearing conditions. Preliminary results showed variations in male and female pupae production between the different temperatures tested (26 to 30°C) and between the two species. These results highlight

the need to keep the temperature constant during the rearing process.



Mosquito rearing in climate-controlled chambers (KBF LQC / KBF LQC-UL (E6)) BINDER) at IPCL.

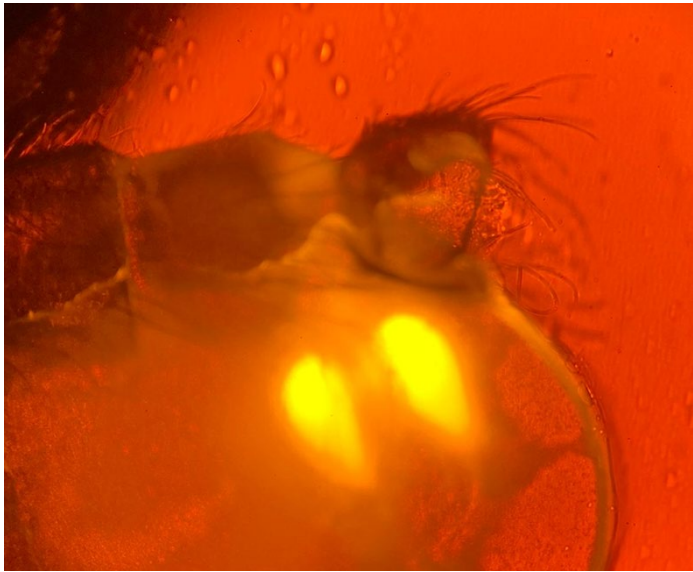
Response of Adult *Aedes* mosquitoes to Gamma Radiation in Different Nitrogen Environments

It has been reported that irradiation of adult mosquitoes is more efficient and results in better male quality than irradiation at the pupal stage. However, the main challenge is to immobilize and compact a large number of adult males for homogenous irradiation with minimal physical damage to the fragile mosquitoes. The use of nitrogen as an anaesthetic during the irradiation of adult *Ae. albopictus* and *Ae. aegypti* was investigated in a recent study. Irradiation in nitrogen (N_2), and in air after being treated with nitrogen (Pre N_2) were compared with irradiation in air. In both species, irradiation in air resulted in higher sterility levels when compared to irradiation in air after immobilization in nitrogen (Pre N_2), and lowest irradiation effects (sterility) were observed in samples immobilized and irradiated in N_2 . Nitrogen treatments showed beneficial effects on irradiated male longevity, revealing the radioprotective effect of anoxia. However, irradiation in nitrogen slightly reduced male flight ability. Overall, these results demonstrated that nitrogen can be useful in adult *Aedes* mass-irradiation. The Pre N_2 treatment is promising as it reduces the immobilization duration and requires a lower dose than an N_2 environment to achieve full sterility but with similar positive effects on male quality.

Rhodamine B Marking of *Anopheles arabiensis* for Competitiveness Trials

Mosquitoes can be fed with Rhodamine B to mark sperm for a variety of mating studies. Feeding *Aedes aegypti* and *Ae. albopictus* with Rhodamine B in sugar solutions is an effective method, for e.g., mating competitiveness studies, where fluorescent sperm can be detected in spermathecae of mated female using a fluorescence microscope. Preliminary studies with *Anopheles arabiensis* have indicated that this species is more susceptible to the Rhodamine toxicity, and significantly lower concentrations are needed as compared to both *Aedes* spp. Furthermore, the marking in treated males persisted for at least ten days, and the fluorescence can also

be detected in the spermathecae of mated females, making this a practical marking method also for this species.



Anopheles arabiensis testes (dissected) filled with Rhodamine B marked sperm.

From the Laboratory to the Field: Long-distance Mass-transport of Sterile Mosquitoes

Operational programmes that use the SIT against *Aedes aegypti* and *Aedes albopictus* may rely on importing significant and consistent numbers of high-quality sterile males. Long-distance mass-transport of chilled sterile males without adverse impact on their survival and quality may contribute to meet this requirement. This study therefore aimed to develop and validate a novel mass-transport method for long distance shipment of sterile male

mosquitoes from the IPCL, Seibersdorf, Austria to Dakar in Senegal.



A prototype of mass-transport box filled with 12 000 chilled sterile male *Aedes* mosquitoes.

Quality parameters of more than 300 000 sterile adult male mosquitoes were assessed in terms of survival rates/recovery rates, flight ability and damage. The novel mass-transport protocol allowed for long-distance shipment of sterile male mosquitoes for up to two days without significant impact on survival (less than 10% of recorded mortality), flight ability and damage. In addition, a recovery time of one day increased sterile male quality in terms of flight ability by more than 20%. The novel long-distance mass-transport protocol may therefore be used to ship sterile males worldwide in SIT programmes.

Reports

11th International Symposium on Fruit Flies of Economic Importance, 13–18 November 2022, Sydney Australia

Hosted by Australia New South Wales Department of Primary Industries and organised in cooperation with the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA), the 11th International Symposium on Fruit Flies of Economic Importance (ISFFEI) was successfully held from 13–18 November 2022 in Macquarie University, Sydney Australia.



Group photo of the 11th International Symposium on Fruit Flies of Economic Importance (13–18 November 2022, Sydney Australia).

The symposium was attended by 355 fruit fly researchers, plant protection officials, fruit industry representatives, fruit fly control companies and exhibitors in the world. Among them, 246 participants from 46 countries joined in the meeting in person, and 109 participants from 33 countries joined virtually. There were 60 oral and 157 poster presentations during the symposium that covered the following 10 sessions: (1) Biology, Ecology, Physiology and Behavior, (2) Taxonomy and Morphology, (3) Genetics and Biotechnology, (4) Chemical Ecology and Attractants, (5) Risk Assessment, Quarantine and Post-harvest, (6) Sterile Insect Technique, (7) Natural Enemies and Biological Control, (8) Other Control Methods and New Developments, (9) Areawide IPM and Action Programs, (10) Social, Economic and Policy Issues of Action Programs.

Many oral and poster presentations covered various aspects related to sterile insect technique (SIT) as a component of area-wide integrated pest management (AW-IPM) against fruit fly pests, such as mass-rearing, field release, genetic sexing strains, quality control, preventive release etc. The success stories on SIT application were shared in the symposium, including the eradication of medfly in Colima, Mexico, the eradication of Queensland fruit fly in Perth, Western Australia, the Medfly programme in Guatemala, the sterile fly preventive release programme in Argentina etc. These highlight that the SIT is a cost-effective, environmentally sustainable and publicly accepted method for the integrated management of fruit fly pests. In addition, some new developments on SIT were also presented during the symposium including the new generation of genetic sexing strains, the simultaneous application of sterile insect and male annihilation techniques to enhance fruit fly management, and the development of the SIT to control the spotted-wing drosophila (SWD) etc.



Opening Ceremony of the 11th International Symposium on Fruit Flies of Economic Importance (Sydney Australia).

Two technique tour visits took place during the symposium 1) the Sydney Markets which is recognised internationally for its world-class fresh produce and community markets. It plays a significant role in the horticultural industry, and the economy of New South Wales and Australia; 2) the Elizabeth Macarthur Agricultural Institute (EMAI) which is a world renowned, premier biosecurity facility that enhances food and fibre production and helps protect the environment.

Announcements

10th International Congress of Dipterology



The 10th International Congress of Dipterology (ICDX) will be held from 16–21 July 2023 in Reno, NV, USA, and is being organized and hosted by the North American Dipterists Society. Dipterology is the Science of Flies, explores the great effect that flies have on our daily lives, as well as their impacts on biodiversity as a whole. To receive updates and information about the ICDX, please sign up to the Dipterists mailing list (<https://lists.dipterists.org/mailman/listinfo/dipterists>) and keep an eye on the ICDX website (<https://dipterists.org/icdx>), which will be continuously updated.

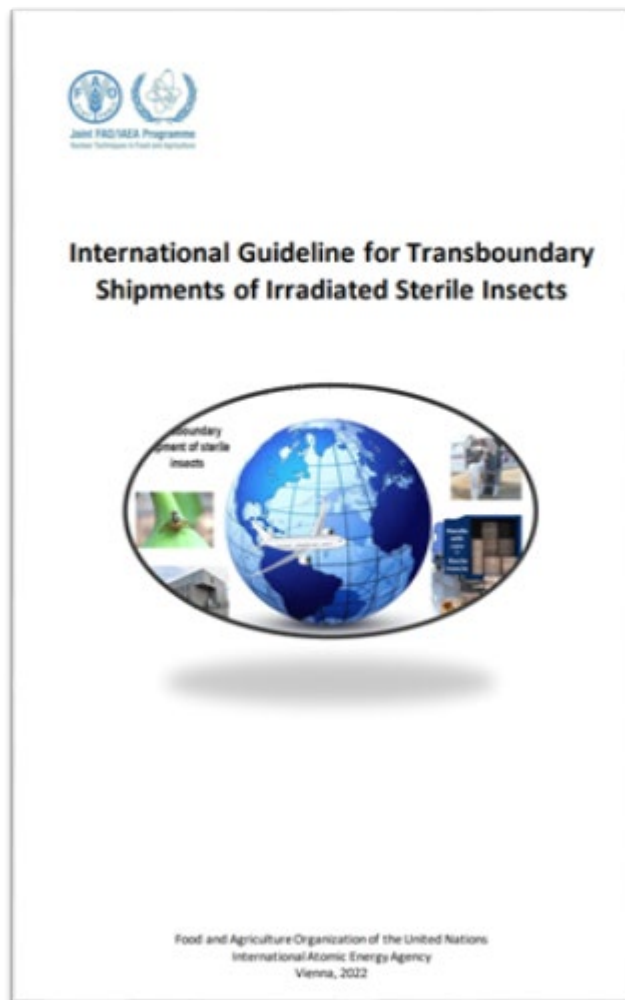
International Guideline for Transboundary Shipments of Irradiated Sterile Insects

This guideline was prepared by a Consultants Group Meeting held in Vienna at the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, from 13 to 17 June 2022 in response to the growing demand from National Plant Protection Organizations (NPPOs), Veterinary Services and Public Health Organizations of FAO and IAEA Member States for alternatives to pesticide use and the increasing interest, also from the private sector, in the application of the sterile insect technique (SIT).

As the SIT becomes more commercial, the need for guarantees that the sterile insects can be safely and legally shipped is essential to encourage financial investments in commercial sterile insect mass-rearing facilities. Also, international regulations are required to reduce the need for independent development of national regulations that may hinder the insect control programmes. This document contains guidelines for transboundary shipment and importation of irradiated sterile insects (either as a consignment in transit or for entry in the country of destination). Producers/shippers and importers of sterile insects may be private businesses as well as government, parastatal, joint venture or international organizations. It is suggested to keep the Insect Pest Control (IPC) Subprogramme informed of any difficulties in compliance with the procedures or gaps in understanding of the procedures. Contact with the IPC Subprogramme can facilitate awareness of new developments in operation procedures available in guidelines and manuals (Contact us: W.R.Enkerlin@iaea.org).

The PDF version of the guideline now is available for download at IPC website

https://www.iaea.org/sites/default/files/2022.transboundary_shipments_of_sterile_insects.pdf



ISO/ASTM 51940:2022-Guidance for Dosimetry for Sterile Insects Release Programs

This document was prepared by ASTM Committee E61 Radiation Processing and by Technical Committee ISO/TC 85, nuclear energy, nuclear technologies and radiological protection.

The purpose of this document is to present information on the use of ionizing energy for the radiation-induced reproductive sterilization of live insects for use in pest management programmes.

The document is intended to serve as a recommendation to be followed when using irradiation technology were approved by an appropriate regulatory authority.

INTERNATIONAL ISO/ASTM STANDARD 51940

Fourth edition
2022-08

Guidance for dosimetry for sterile insects release programs

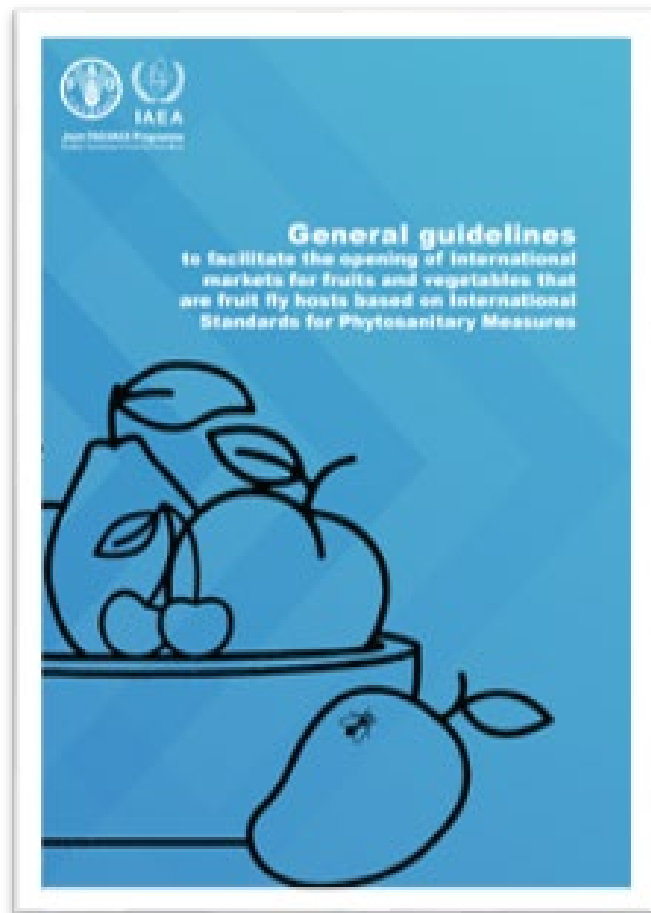
*Lignes directrices de la dosimétrie pour des programmes de lâchers
d'insectes stériles*

It is not to be construed as a requirement for the use of irradiation nor as a required code of practice. While the use of irradiation involves certain essential requirements to attain the objective of the treatment, some parameters can be varied in optimizing the process.

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

The guideline was prepared under the framework of the IAEA Technical Cooperation Project RLA5082 Strengthening Food Security through Efficient Pest Management Schemes Implementing the Sterile Insect Technique as a Control Method. It aims for a better understanding of the factors that should be considered when establishing fruit and vegetables trade, and how to maximize the potential for international standards to facilitate safe trade.

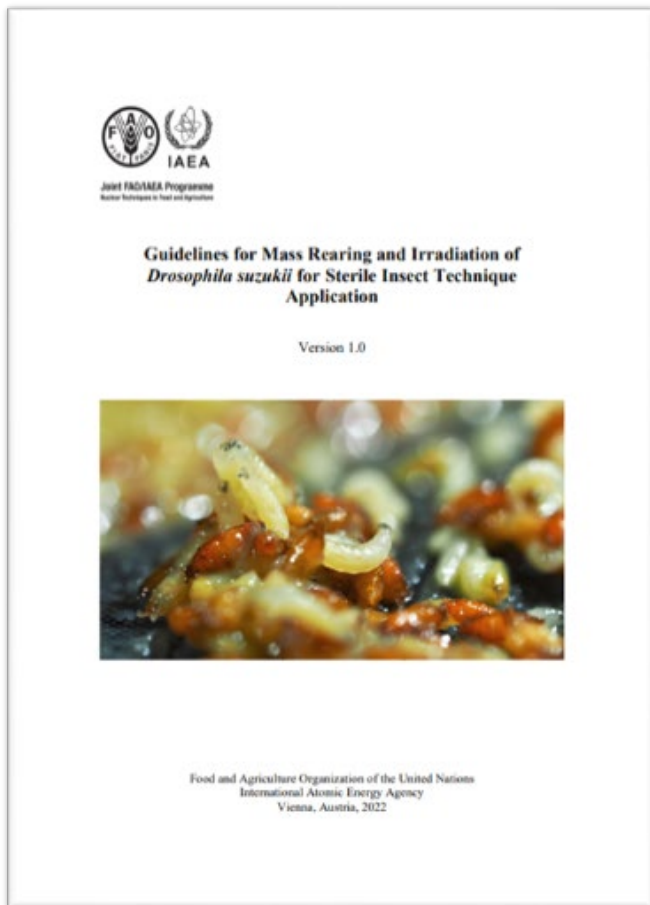
The key to opening markets in the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement) and the International Plant Protection Convention (IPPC) framework, is to focus on the pest risk, and especially on all the factors contributing to risk and mitigating risk.



There is often a tendency to begin with legacy concepts and requirements that were based on assumptions of high risk rather than begin with a fresh view of the evidence and an objective analysis of the risk. By breaking from the dogma of historical designs to embrace the opportunities for innovation created by the WTO-SPS-IPPC framework, researchers and regulators are able to create regulatory designs that more closely align with the concept of rational relationship and conform to relevant standards.

The PDF version of this Guideline is now available for download at IPC website <https://www.fao.org/documents/card/en/c/cc0361en>.

Guidelines for Mass Rearing and Irradiation of *Drosophila suzukii* for Sterile Insect Technique Application



The Guideline describes the standard mass-rearing protocols and sterilization procedures currently used at the Insect Pest Control Laboratory (IPCL). The first part of the document, it describes the mass-rearing oviposition system used at the IPCL and detail the different steps necessary to domesticate and maintain a laboratory colony of spotted wing *Drosophila* (SWD), *Drosophila suzukii*, in particular egg collection, larval rearing, and pupae collection.

The second part, it presents the pupal irradiation procedure, as well as the method to measure adult sterility level. All research presented in the guideline have been developed using a single colony established in 2014 from pupae sent by the Agricultural Entomology Research Unit of the Edmund Mach Foundation (San Michele all'Adige, Trentino, Italy). In the future, an additional document will present the quality control procedures developed at the IPCL, including routinary testing and mating compatibility and competitiveness testing.

The PDF version of the Guideline is now available at IPC website <https://www.iaea.org/sites/default/files/massrearing-and-irradiation-swd.pdf>.

In Memoriam

John Mumford (1953–2023)

It is with great sadness that we have to inform that John Mumford passed away unexpectedly on 29 December 2022 at his home in Windsor, United Kingdom.



John received his PhD degree in Applied Entomology from the Imperial College London in 1978 and rose rapidly to be appointed a lecturer there the following year. Starting as a lecturer in crop protection, he became the Director of the Centre for Environmental Policy and since 2002 Professor of Natural Resource Management.

He was an international authority on economic, decision and policy analyses for biosecurity, agricultural pest and vector management, fisheries, and resource management risks. He led collaborative missions throughout the world to determine environmental risk management research, to provide training and define implementation priorities. He was a member of advisory bodies for UK and UN technical cooperation agencies in agricultural and environmental development.

For many years (since 1992) he closely collaborated with the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture in various capacities. As an external advisor for the IAEA Standing Advisory Group on Nuclear Applications, the advisory body of the IAEA Department of Nuclear Sciences and Applications, as a consultant on sterile insect technique (SIT) economic feasibility assessments for IAEA and FAO Member States, as an invited speaker in international symposiums and conferences, and contributed with many scientific articles, book chapters and technical documents on the economics of area-wide SIT interventions. John, together with his research team and students at the Imperial College in London, developed a benefit-cost analysis scheme to measure the return on investment of area-wide SIT implementation. The scheme was transformed into a Benefit–Cost Analysis Model to support decision making to undertake large-scale SIT interventions.

John excelled in his professional career with many outstanding contributions in the fields of biology and environment. He was an intellectual leader including in the areas of integrated pest management and environmental policy. He trained and provided academic and professional guidance to many bachelor and postgraduate students worldwide. His legacy will keep on living in the hearts and minds of countless students and professionals around the world. He will be greatly missed.

A memorial will be held for John on 2 March in Windsor, United Kingdom. For more detailed information on the memorial please kindly contact Mrs Megan Quinlan (m.quinlan@imperial.ac.uk).

Hugh John Barclay (1941–2022)

With great sadness we have to inform you that Hugh Barclay, retiree from the Pacific Forestry Centre, Canadian Forest Service passed away on 15 October 2022. Hugh has collaborated with the Insect Pest Control Subprogramme, mainly on developing models for assessing the male annihilation of *Bactrocera* spp. with methyl eugenol baits, integration of male annihilation and the Simultaneous release of methyl eugenol-exposed *Bactrocera* spp. sterile males, the minimum size area for an area-wide integrated pest management program and a dynamic population model for tsetse (Diptera: Glossinidae) area-wide integrated pest management.



With the departure of Hugh, we have lost a remarkable scientist and friend. Those who knew him will remember him for his friendship, his scientific endeavour and his kindness.

Other News

Artificial Intelligence Performs Key Step in Fruit Fly Management

Two of the world’s most damaging pests are the Mediterranean fruit fly (*Ceratitis capitata*) and the Mexican fruit fly (*Anastrepha ludens*), causing billions of dollars in damage to agriculture. Fortunately, the sterile insect technique (SIT) is currently used as part of area-wide integrated management programmes to control these flies in certain regions of the world.



The Mexican fruit fly (*Anastrepha ludens*) is one of the world’s most damaging insect pests. (Photo by Andrés Díaz Cervantes).

The irradiation process in SIT is key to its success. For tephritid flies, irradiation is usually carried out a couple of days before the pupae emerge as adults. If pupae are irradiated too soon or too late in their development process, this can lead to problems in mobility and behaviour as adults. However, even during controlled conditions, pupae can vary in their development time. Thus, one of the tests that are carried out pre-irradiation is to determine the physiological age of the pupae.

Currently, at these fruit fly factories throughout the world, technicians must determine the correct time to irradiate by taking a sample of pupae, removing the pupal case to expose the eyes, and then checking the eye colour against a colour chart. This can be laborious and prone to human error, as it depends on the skill, experience, and expertise of the technician, as well as natural biases in colour interpretation. The technicians can get tired from this repetitive work, while sick days and vision problems could also cause variations in the correct determination.

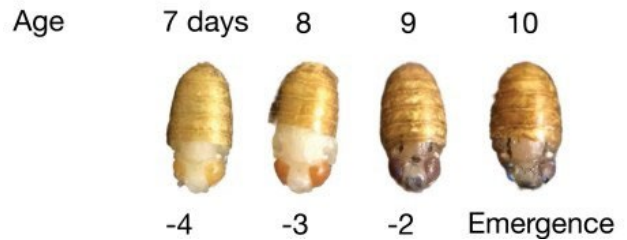
At the Universidad Veracruzana, in collaboration with the Secretary of Agriculture of Mexico (Programa Operativo de Moscas, DGSV-SENASICA), we teamed up with experts in artificial intelligence to develop methods based on algorithms that can accurately determine the age of a pupa from a digital image captured with a common mobile device. We share our results in a new article published September 2022 in the *Journal of Economic Entomology*.

For this, and as part of his Ph.D. at the Facultad de Ciencias Agrícolas of the Universidad Veracruzana, Iván González-López, took photographs of the exposed eyes of pupae of both Mediterranean fruit flies and Mexican fruit flies. We chose pupae that still had a few days to emerge and deliberately took rough photographs that did not have perfect lighting conditions or focus. In fact, they were taken quickly and with a mobile phone.

Anastrepha ludens



Ceratitis capitata



Anastrepha ludens (Tapachula-7) and *Ceratitis capitata* (Vienna-8) pupae according to physiological age and days until emergence. Emergence refers to pupae emerging or about to emerge. Both species are irradiated at -2 d before emergence. (Image originally published in González-López et al 2022, *Journal of Economic Entomology*).

Then, as a part of her master’s research at the Laboratorio Nacional de Informática Avanzada in Xalapa Veracruz, Georgina Carrasco processed the images with a program that was trained to detect the eye area in the photograph and crop it. Afterward, using the correct answers from a technician at the factory, another algorithm was trained through a supervised machine-learning method known as transfer learning, to accurately determine the age of the pupae.

We found that algorithms based on a neural network architecture known as Inception v1 correctly identified the physiological age of maturity at two days before emergence, with a 75 percent accuracy for the Mexican fruit fly and 83 percent for Mediterranean fruit fly, respectively. This method is not perfect for sure, and it still requires a technician to dissect the pupae and take photographs, but it is a promising approximation of how supervised machine learning and artificial intelligence can be used to help uncertainty in decisions about when to irradiate. The level of accuracy may also be improved as more pictures are taken and provided for the algorithm to learn from.

The next steps will be to develop software that could easily be used by technicians as well as to train these algorithms with other tephritid pest species currently controlled through SIT. Certainly, it highlights that there can be some exciting collaborations between entomologists and artificial intelligence researchers.

Source: *Entomology Today*, by Diana Pérez-Staples and Horacio Tapia-McClung, <https://entomologytoday.org/2022/09/28/artificial-intelligence-performs-key-step-fruit-fly-management-sterile-insect-technique/>.

New Findings of *Bactrocera dorsalis* in Italy

Bactrocera dorsalis (Diptera: Tephritidae – EPPO A1 List) was detected for the first time in Southern Italy (Salerno and Napoli provinces, Campania region) during an official survey in April 2018 (EPPO RS 2018/215, RS 2019/096). Official monitoring has been conducted since, but only a few specimens have been trapped in the following years. These findings were not considered as outbreaks but as incursions associated with fruit import.



Female *Bactrocera dorsalis* depositing eggs in a ripe fruit (Photo credit: Ana Rodriguez FAO/IAEA).

In 2022, *B. dorsalis* was trapped in June in Campania region (municipality of Palma Campania) and further specimens were trapped in August and September in this municipality and once in San Gennaro Vesuviano

The origin of these findings is not known but it is noted that a large proportion of the population in the area originates in Asia, and it is considered that findings may be related to infested fruits brought back by travellers.

The NPPO of Italy recently provided an update: in the second half of September, 525 adults (5 females and 520 males) were caught in 36 traps set in Palma Campania and San Gennaro Vesuviano; in the first week of October, 310 adults were caught (305 males and 5 females) including in the neighbouring municipalities of Ottaviano (2 males) and Nola (4 males).

In addition, fruit sampling showed that some adults of *B. dorsalis* emerged from fruits collected on the ground within a 10 m radius of the traps in early September (2 females from oranges, and 2 females and 3 males from a peach).

A delimited area has been established and includes part of the territories of the four municipalities and a buffer zone of 7.5 km. Measures applied include harvesting and destruction of fruits, prohibition of fruit movement out of the demarcated area, insecticide treatments, intensification of surveys with trapping and soil analysis.

The pest status of *Bactrocera dorsalis* in Italy is officially declared as: Transient, actionable, under surveillance.

Source: *eppc.int*, EPPO Reporting Service no. 09 – 2022; Num. article: 2022/188 <https://gd.eppo.int/reporting/article-7419> and no. 10 – 2022; Num. article: 2022/211 <https://gd.eppo.int/reporting/article-7442>.

Relevant Published Articles

Harmonic radar tracking of individual melon flies, *Zeugodacus cucurbitae*, in Hawaii: Determining movement parameters in cage and field settings

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Abstract

Tephritid fruit flies, such as the melon fly, *Zeugodacus cucurbitae*, are major horticultural pests worldwide and pose invasion risks due primarily to international trade. Determining movement parameters for fruit flies is critical to effective surveillance and control strategies, from setting quarantine boundaries after incursions to development of agent-based models for management. While mark-release-recapture, flight mills, and visual observations have been used to study tephritid movement, none of these techniques give a full picture of fruit fly movement in nature. Tracking tagged flies offers an alternative method which has the potential to observe individual fly movements in the field, mirroring studies conducted by ecologists on larger animals. In this study, harmonic radar (HR) tags were fabricated using superelastic nitinol wire which is light (tags weighed less than 1 mg), flexible, and does not tangle. Flight tests with wild melon flies showed no obvious adverse effects of HR tag attachment. Subsequent experiments successfully tracked HR tagged flies in large field cages, a papaya field, and open parkland. Unexpectedly, a majority of tagged flies showed strong flight directional biases with these biases varying between flies, similar to what has been observed in the migratory butterfly *Pieris brassicae*. In field cage experiments, 30 of the 36 flies observed (83%) showed directionally biased flights while similar biases were observed in roughly half the flies tracked in a papaya field. Turning angles from both cage and field experiments were non-random and indicate a strong bias toward continued “forward” movement. At least some of the observed direction bias can be explained by wind direction with a correlation observed between collective melon fly flight directions in field cage, papaya field, and open field experiments.

However, individual mean flight directions coincided with the observed wind direction for only 9 out of the 25 flies in the cage experiment and half of the flies in the papaya field, suggesting wind is unlikely to be the only factor affecting flight direction. Individual flight distances (meters per flight) differed between the field cage, papaya field, and open field experiments with longer mean step-distances observed in the open field. Data on flight directionality and step-distances determined in this study might assist in the development of more effective control and better parametrize models of pest tephritid fruit fly movement.

The full paper was published in: *PLoS ONE* 17(11):e0276987. <https://doi.org/10.1371/journal.pone.0276987>

The dacine fruit flies (Diptera: Tephritidae: Dacini) of Oceania

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Abstract

A key, an annotated checklist with detailed distribution, biological and host information, and color photographic plates are provided for the 91 species of dacine fruit flies (Diptera: Tephritidae: Dacini) known to occur in Oceania. *Dacus virgatus* Coquillett, previously a synonym of *Bactrocera psidii* (Froggatt), is instead considered a **junior synonym** of *B. facialis* (Coquillett). The species originally described in 1971 as *Dacus (Asiadacus) perpusillus* Drew, later reassigned as *Bactrocera (Sinodacus) perpusilla* (Drew) and in recent years as *Zeugodacus (Sinodacus) perpusillus* (Drew) actually belongs to genus *Dacus*, and is transferred back to *Dacus*, but to the subgenus *Neodacus*, **restored combination**. The presence of *B. reduunca* (Drew) is recorded for the first time in New Caledonia. New male lure records include isoeugenol and dihydroeugenol for both *B. neoxanthodes* Drew and Romig and *B. quadrisetosa* (Bezzi) and zingerone for *Dacus taui* (Drew and Romig), all in Vanuatu.

The full paper was published in: *Insecta Mundi* (2022) 0948: 1–167, <https://journals.flvc.org/mundi/article/view/131965/135549>.

Does severe hypoxia during irradiation of *Aedes aegypti* pupae improve sterile male performance?

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Abstract

Background: The yellow fever mosquito, *Aedes aegypti*, vectors several pathogens responsible for human diseases. As a result, this mosquito species is a priority for control by mosquito control districts in Florida. With insecticide resistance development becoming a concern, alternative control strategies are needed for *Ae. aegypti*. Sterile insect technique (SIT) is an increasingly popular option that is being explored as a practical area-wide control method. However, questions about sterile male performance persist. The objectives of this study were to determine the extent to which hypoxia exposure prior to and during irradiation effects the longevity, activity and mating competitiveness of sterile male *Ae. aegypti*.

Methods: Male longevity was monitored and analyzed using Cox regression. Mosquito activity was recorded by an infrared beam sensor rig that detected movement. Competing models were created to analyze movement data. Fecundity and fertility were measured in females mated with individual males by treatment and analyzed using oneway ANOVAs. Mating competition studies were performed to compare both hypoxia and normoxia treated sterile males to fertile males. Competitiveness of groups was compared using Fried's competitiveness index.

Results: First, we found that subjecting *Ae. aegypti* pupae to 1 h of severe hypoxia (< 1 kPa O₂) did not directly increase mortality. One hour of hypoxia was found to prevent decreases in longevity of irradiated males compared to males irradiated in normoxic conditions. Exposure to hypoxia prior to irradiation did not significantly improve activity of sterile males except at the highest doses of radiation. Hypoxia did significantly increase the required dose of radiation to achieve > 95% male sterility compared to males irradiated under normoxic conditions. Males sterilized after an hour in hypoxic conditions were significantly more competitive against fertile males compared to males irradiated under normoxic conditions despite requiring a higher dose of radiation to achieve sterility.

Conclusions: Hypoxia was found to greatly improve key performance metrics in sterile male *Ae. aegypti* without any significant drawbacks. Little work other than increasing the target dose for sterility needs to be conducted to incorporate hypoxia into SIT programs. These results suggest that SIT programs should consider including hypoxia in their sterile male production workflow.

Keywords: Sterile insect technique, Hypoxia, *Aedes*, Mating competitiveness

The full paper was published in: *Parasites & Vectors* (2022) 15:446, <https://doi.org/10.1186/s13071-022-05577-0>

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