



Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Insect & Pest Control Newsletter



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To Our Readers



Third instar larvae of Aedes aegypti being mass-reared in response to requests to develop the SIT package for vectors of dengue and chikungunya, mosquitoes-transmitted viral diseases that have been rapidly spreading in the world. Equipment and protocols are being developed and validated for the efficient mass-rearing, irradiation and release of Aedines and Anophelines that will also benefit other genetic approaches under development to control mosquitoes (photo credit Jesus Reyes).

Despite the amazing progress made in science and technology during the last hundred years, humankind still faces significant challenges in combating pest insects, such as mosquitoes that are the vectors of major pathogens (arboviruses and bacterial as well as eukaryotic microorganisms). These pathogenic microorganisms cause infectious diseases resulting in severe morbidity or lethality.

According to the World Health Organization (WHO), there are over 200 million cases of malaria resulting in more than 600 000 deaths annually, mainly very young children. The great majority of malaria deaths occur in sub-Saharan Africa. Currently, malaria transmission occurs in about 100 countries putting about 3.4 billion people at risk (World Malaria Report, 2013).

Similarly, around 400 million people contract every year a dengue infection of which about 500 000, mainly children require hospitalization; it is estimated that 2.5% of them die. Dengue has spread globally during the last years and currently over 3 billion people are at risk in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. The majority of dengue cases are reported in American, Southeast Asian and the Western Pacific regions. Recently another viral mosquito-borne disease, chikungunya, has been spreading rapidly. It is a disease that causes severe chronic joint pain in patients across the globe.

In the absence of effective vaccines and drugs, these mosquito-transmitted diseases pose an enormous economic and social burden worldwide and their incidence has increased drastically in recent years. In addition, the traditional chemical-based vector control strategies are facing serious challenges due to increased resistance of mosquitoes to the used insecticides and increased public concern of insecticide use in urban areas. Based on these facts, novel methods and complementary approaches are required to manage mosquito populations in an effective and more environmentally friendly and sustainable way.

Given the burden of these mosquito-borne infectious diseases, there are numerous requests by Member States to develop and implement the sterile insect technique (SIT) as a component of area-wide integrated pest management approaches to control mosquito populations. In response to these requests and the GC resolution GC(58)/RES/13, the Joint FAO/IAEA Insect Pest Control Subprogramme has started developing a complete "SIT package" against mosquito species with emphasis on the malaria vector species *Anopheles arabiensis* and the dengue and chikungunya vector species *Aedes aegypti* and *Ae. albopictus*.

In-house research at the FAO/IAEA Insect Pest Control Laboratory and at collaborating institutions is focused on all aspects of a mosquito SIT package. Rearing and handling protocols, diets and equipment have been developed for the mass-rearing of both *Anopheles* and *Aedes* species, including methods for egg storage and quantification. Irradiation studies have also been carried out to determine the

dose required for complete male sterility without affecting male mating competitiveness.

Under the IAEA Technical Cooperation Programme we have been transferring all the above developments to a number of Member States, while we keep optimizing and standardizing them. In addition, semi-field and field tests have shown that sterilized males can successfully compete with non-treated or wild males for mating with females. It is worth noting that SIT field trials have been carried out or have just started in Mauritius, Italy, Indonesia and Sudan, and that other countries are planning to start trials.

The SIT can be based on bisexual (male and female) releases, although it has been shown in fruit flies that its cost-effectiveness and efficiency are increased if only sterile males are released. However, in the case of mosquito SIT (or any other relevant mosquito population suppression method), releasing only males is an absolute prerequisite, more to avoid any possibilities of disease transmission by released females than increased effectiveness and cost efficiency.

In view of this need, the Joint FAO/IAEA Insect Pest Control Subprogramme initiated about two years ago a Coordinated Research Project (CRP D4.40.01) on "*Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes*". The 2nd Research Coordination Meeting (RCM) took place 9-13 March 2015 at the Moscamed facilities in Juazeiro, Brazil.

The results of this CRP are so far encouraging and indicate that, although challenging, it is possible to develop methods for the complete elimination of females and their application at large scale operational programmes. In the meantime, however, and in the absence of an efficient and robust sex separation method, the safest solution for vector population suppression is the integration of the SIT with other genetic approaches such as *Wolbachia*-induced cytoplasmic incompatibility (incompatible insect technique, IIT) and pathogen interference. This combined approach has been developed by the FAO/IAEA Insect Pest Control Laboratory and collaborators to support recently started field trials to control populations of *Ae. albopictus* in China.

The combination of SIT and IIT strategies allows complete sterilization of females with low irradiation doses, thus eliminating the risk of the accidental release of fertile females. This avoids the loss of the IIT tool and the replacement of the wild population. Such a replacement may have unforeseen consequences, for example with regards to its vector capacity. However, once the *Wolbachia*-infected females are released, the population replacement can no longer be reversed. Therefore, such a permanent and self-sustaining establishment in space and time of a foreign entity requires regulatory approvals in some countries and is not acceptable in many others.

Also in the case of transgenic mosquito releases, potential risks, such as the presence of transgenic F₁ or biting fe-

males in the field, as well as transgene horizontal transfer can be avoided by an irradiation treatment that ensures complete sterility of the released males and females. The North American Plant Protection Organization (NAPPO) has such a requirement for the release of transgenic strains to control insect pests of agricultural importance.

Recently, the planned release of transgenic mosquitoes in the Florida Keys, USA has been the subject of a public outcry. The perception that mosquito disease vectors can only be controlled either with insecticides or with transgenic insects is prevailing with the public. This is clearly a misconception as there are other options being developed and validated as reported above. There is clearly no "silver bullet" for managing mosquito vector populations. All available tools and strategies are potentially useful, not as stand-alone tactics, but as part of an integrated approach. Nevertheless issues of stability, sustainability and biosecurity have to be addressed.

Not only the SIT, but also other genetic approaches to mosquito control, will require protocols and methods for handling, packaging, transporting and releasing the sterile males. In addition, robust and accurate methods for post-release monitoring to assess the dispersal and survival of released males as well as the density of the native target population will be required. This information will be essential for the optimization of the release operations and the overall evaluation of the population suppression programme. In response to this gap and to address these needs, we are initiating a CRP (D4.40.02) on "*Mosquito Handling, Transport, Release and Male Trapping Methods*" and a call for participants has been opened this year (for this as well as all other CRPs: <http://www-naweb.iaea.org/nafa/ipc/crp/new-crps-ipc.html>).

Despite the focus of this note being on aspects relating primarily to mosquito control, you will find also the traditional sections of the Newsletter with the work on other pest insects to which you have become accustomed over the years. We would also like to draw your attention to an animated infographic

<http://www-naweb.iaea.org/nafa/resources-nafa/SIT-233stream32.mp4>) that was produced in collaboration with graphic animation experts at FAO headquarters to make the SIT more easily understandable to the wider general public.



FAO animated infographic to make the Sterile Insect Technique more easily understandable to the wider general public.

We thank our collaborators in many parts of the world for your support and many exiting and very productive interactions. We hope you enjoy browsing the Newsletter and look forward to any thoughts you may want to communicate.

Jorge Hendrichs
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Forthcoming Events (2015-2016)

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

First RCM on Comparing Rearing Efficiency and Competitiveness of Sterile Male Strains Produced by Genetic, Transgenic or Symbiont-based Technologies. 6–10 July 2015, Vienna, Austria.

Third RCM on Use of Symbiotic Bacteria to Reduce Mass-rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application. 26–30 October 2015, Antigua, Guatemala.

First RCM on Mosquito Handling, Transport, Release and Male Trapping Methods. 23–27 November 2015, Vienna, Austria.

Second RCM on Dormancy Management to Enable Mass-rearing and Increase Efficacy of Sterile Insects and Natural Enemies. 18–22 April 2016, Stellenbosch, South Africa.

Third RCM on Enhancing Vector Refractoriness to Trypanosome Infection. 6–10 June 2016, Lyon, France.

First RCM on Improved Field Performance of Sterile Male Lepidoptera to Ensure Success in SIT Programmes. 4–8 July 2016, Durban, South Africa.

Third RCM on Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes. 10–14 October 2016, Pereybere, Mauritius.

II. Consultants and Expert Meetings

FAO/IAEA Consultants Meeting on Greenhouse and Other Confined Pest SIT. 14–18 March 2016, Vienna, Austria.

III. Other Meetings/Events

FAO/IAEA Regional Training Course on Fruit Fly Monitoring and Suppression Including MAT and SIT for Indian Ocean (under Regional TC Project RAF5062). 29 June–3 July 2015, Reduit, Mauritius.

FAO/IAEA Regional Meeting on SIT Application, Including Rearing, Irradiation and Release of Fruit Flies (under Regional TC Project RAF5062). 1–3 July 2015, Reduit, Mauritius.

FAO/IAEA Regional Training Course on Free Open Source Software for GIS and Data Management Applied to Fruit Flies in the Balkans and the Eastern Mediterranean (under Regional TC Project RER5020). 27–31 July 2015, Vienna, Austria.

FAO/IAEA Interregional Training Course on The Use of the Sterile Insect and Related Techniques for the Integrated Area-wide Management of Insect Pests (under Interregional TC Project INT5151). 3–28 August 2015, Metapa de Dominguez, Chiapas, Mexico and Antigua / El Pino, Guatemala.

Meeting of the Technical Panel on Phytosanitary Treatments (TPPT), International Plant Protection Convention, FAO. 31 August–4 September 2015, Fukushima, Japan.

FAO/IAEA Regional Training Course on The Use of Population Genetics and GIS to Identify Isolated Tsetse Populations, (under Regional TC Project RAF5070). 21 September–2 October 2015, Addis Ababa, Ethiopia.

FAO/IAEA Expert Meeting on Harmonization of Post-factory Product Quality Control for fruit fly SIT. 28 September–2 October 2015, Vienna, Austria.

Meeting of the Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies (TPFF), International Plant Protection Convention. 19–23 October 2015, Vienna, Austria.

Workshop on Microbial and Processing Criteria for Industrial Production of Probiotics or Bacteria as Source of Protein to Improve Fruit Fly Quality and SIT Efficiency. 23–25 October 2015, Guatemala City, Guatemala.

FAO/IAEA Regional Training Course on Taxonomy and Identification of Fruit Fly Pest Species for Southeast Asia (under Regional TC Project RAS5067). 16–20 November 2015, Brisbane, Australia.

Third Meeting of the Tephritid Workers of Europe, Africa and the Middle East (TEAM), 11–14 April 2016, Stellenbosch, South Africa.

Workshop on Dormancy Management to Enable Insect Mass-rearing. 15–17 April 2016, Stellenbosch, South Africa.

Workshop on Bioinformatics Approaches for Microbiota Profiling Based on Amplicon Sequencing Data. 30 May–1 June 2016, Lyon, France.

Workshop on Tissue Localization and Analysis of Insect Endosymbionts by Fluorescence Microscopy: Theoretical Background and Practical Applications. 2–4 June 2016, Lyon, France.

Workshop on Standardized Sampling and Bioassay Methods for Assessing Field Performance of Sterile Male Lepidoptera. 2–3 July 2016, Durban, South Africa.

First Meeting of the Tephritid Workers of Asia, Australia, and Oceania. (TAAO), 15–18 August 2016, Kuala Lumpur, Malaysia.

Ninth Meeting of the Tephritid Workers of the Western Hemisphere (TWWH), tentatively for October 2016, Buenos Aires, Argentina.

Third FAO/IAEA International Conference on Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques. 22–26 May 2017, Vienna, Austria.

Past Events (2014-2015)

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

Final RCM of CRP on Development and Evaluation of Improved Strains of Insect Pests for SIT. 7–11 April 2014, Capri, Italy.

Second RCM of CRP on Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application. 6–10 May 2014, Bangkok, Thailand.

Final RCM of CRP on Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control. 2–6 June 2014, Kelowna, Canada.

Final RCM of CRP on Development of Generic Irradiation Doses for Quarantine Treatments. 2–6 June 2014, Vienna, Austria.

First RCM of CRP on Dormancy Management to Enable Mass-Rearing and Increase Efficacy of Sterile Insects and Natural Enemies. 21–25 July 2014, Vienna, Austria.

Second RCM of CRP on Enhancing Vector Refractoriness to Trypanosome Infection. 1–5 December 2014, Addis Ababa, Ethiopia.

Second RCM on Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes. 9–13 March 2015, Juazeiro, Brazil.

Final RCM on Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade. 1–5 June 2015, Saint Pierre, La Réunion, France.

II. Consultants and Expert Meetings

FAO/IAEA Consultants Meeting on New Methods for the Detection and Quantification of Irradiation in Intercepted Insects. 10–14 March 2014, Vienna, Austria.

FAO/IAEA Consultants Meeting on Process Control for Fruit Fly Rearing Facilities that Mass-Produce Insects for Sterilization. 13–15 May 2014, Bangkok, Thailand.

FAO/IAEA Consultants Meeting on Developing Sterile Mosquito Transport and Aerial Release Methods. 8–12 December 2014, Vienna, Austria.

Consultants Meeting on Mosquito Male Trapping Methods to Monitor the Efficacy of SIT Programme in the Field. 16–20 February 2015, Vienna, Austria.

Consultants Meeting on Improved Field Performance of Sterile Moths to Enhance SIT Application. 13–17 April 2015, Vienna, Austria.

Consultants Meeting on A Generic Approach for the Development of Genetic Sexing Strains for SIT Applications. 1–5 June 2015, Vienna, Austria.

III. Other Meetings/Events

FAO/IAEA Sub-regional Assessment Meeting of the Regional TC Project RAS5059 on Supporting Area-wide Integrated Pest Control of Native and Exotic Flies in the Middle East Subregion, Incorporating the Sterile Insect Technique. 25–26 February 2014, Vienna, Austria.

FAO/IAEA Regional Workshop to Present Respective Experiences with Fruit Flies and Synergise Future Activities in the Balkans and the Eastern Mediterranean (under Regional TC Project RER5020). 25–27 March 2014, Vienna, Austria.

Ninth Session of the Commission on Phytosanitary Measures, International Plant Protection Convention, FAO. 31 March–4 April 2014, Rome, Italy.

FAO/IAEA Regional Training Course on Taxonomy and Identification of Fruit Fly Pest Species Exotic to the Middle East (under Regional TC Project RAS5059). 31 March–4 April 2014, Seibersdorf, Austria.

Workshop on Characterization of Symbionts of Fruit Flies of Economic Importance via Bioinformatic Approaches. 4–5 May 2014, Bangkok, Thailand.

9th International Symposium on Fruit Flies of Economic Importance. 12–16 May 2014, Bangkok, Thailand.

FAO/IAEA Regional Training Course on Free Open Source Software for GIS and Data Management Applied to Tsetse and Trypanosomosis Control Programmes. 12–23 May 2014, Addis Ababa, Ethiopia.

FAO/IAEA First Coordination Meeting of the Regional TC Project RLA5067 on Supporting Capacity Building for Evaluation of Feasibility of a Progressive Control Programme for New World Screwworm. 2–6 June 2014, Panama City, Panama.

IAEA Meeting on Thematic Plan for the Development and Application of the Sterile Insect Technique (SIT) and Related Genetic and Biological Control Methods for Disease Transmitting Mosquitoes, 16–20 June 2014, Vienna, Austria.

FAO/IAEA Regional Training Course on Use of GIS for Area-Wide Fruit Fly Programmes in Indian Ocean (under Regional TC Project RAF5062). 18–22 August 2014, Zanzibar, United Republic of Tanzania.

FAO/IAEA Regional Training Course on Fruit Fly Monitoring and Suppression including MAT and SIT for South-east Asia (under Regional TC Project RAS5067). 15–19 September 2014, Bandung, Indonesia.

FAO/IAEA Regional Training Course on Mass-Rearing and SIT-Related Activities for the Control of *Aedes* Mosquitoes (under Regional TC Project RAS5066). 22–26 September 2014, Juazeiro, Brazil.

FAO/IAEA Regional Training Course on Taxonomy and Identification of Fruit Fly Pest Species Exotic to the Balkans and Eastern Mediterranean (Regional TC Project RER5020). 13–17 October 2014, Tervuren, Belgium.

FAO/IAEA Regional Training Course on Diagnosis of the New World Screwworm (under Regional TC Project RLA5067). 27–31 October 2014. Pecora, Panama.

Expert Consultation on Phytosanitary Treatments for the *Bactocera dorsalis* Complex, International Plant Protection Convention, FAO. 1–5 December 2014, Okinawa, Japan.

FAO/IAEA Coordination Meeting of the West Africa Regional TC Project RAF5061 on Supporting Capacity Building and a Feasibility Study on Control of Fruit Flies of Economic Significance in West Africa. 10–12 December 2014, Vienna, Austria.

FAO/IAEA Regional Training Course on Free Open Source Software for GIS and Data Management applied to tsetse and trypanosomosis control programmes, French edition (under the Regional TC Project RAF5070). 19–30 January 2015, Vienna, Austria.

FAO/IAEA Coordination Meeting of the Africa Regional TC Project RAF5069 on Supporting a Feasibility Study to Eradicate Tsetse from Southern Mozambique, South Africa and Swaziland. 9–10 February 2015, Pretoria, South Africa.

FAO/IAEA Regional Training Course on Fruit Fly Biocontrol in West Africa (under Regional TC Project RAF5061). 2–6 March 2015, Nairobi, Kenya.

Tenth Session of the Commission on Phytosanitary Measures, International Plant Protection Convention, FAO. 16–20 March 2015, Rome, Italy.

FAO/IAEA First Meeting for Central America – Mexico to Strengthen Surveillance and Response to Outbreaks of New World Screwworm (COPEG) (under the Regional TC Project RLA5067). 6–8 May 2015, Panama City, Panama.

Technical Cooperation Field 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 six major topics, namely:

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

Country	Project Number	Title National Projects	Technical Officer
Angola	ANG5012	Supporting Feasibility Studies for using Sterile Insect Techniques as part of Area-Wide Integrated Pest Management for Control of Tsetse Flies (<i>G. morsitans centralis</i>)	Rafael Argiles
Burkina Faso	BKF5012	Collecting Baseline Data and Implementing Fruit Fly Suppression in Mango Fruit	Rui Cardoso Pereira
Costa Rica	COS5030	Supporting Biological Control of Stable Flies (<i>Stomoxys calcitrans</i>) through the Use of Parasitoids Reproduced on Fruit Flies	Jesús Reyes
China	CPR5020	Integrating the Sterile Insect Technique (SIT) for Area-Wide Integrated Pest Management of Tephritid Fruit Flies	Rui Cardoso Pereira
Ethiopia	ETH5018	Contributing to the Creation of Sustainable Tsetse Free Areas	Rafael Argiles Andrew Parker
Guatemala	GUA5017	Using the Sterile Insect Technique (SIT) to Establish Fruit Fly Low Prevalence Pilot Areas and to Assess it as an Alternative for the Control of the Sugarcane Borer in Pilot Areas	Jesús Reyes
Honduras	HON5006	Using Sterile Insect Technique (SIT) to Obtain Recognition as a Mediterranean Fruit Fly Free Area in the Aguan River Valley	Jesús Reyes
Israel	ISR5019	Supporting a Feasibility Study for the Implementation of Leafminer (<i>Liriomyza</i> spp) Sterile Insect Technique Combined with Biological Control under Greenhouse Conditions	Jesús Reyes
Libya	LIB5011	Enhancing Area-Wide Integrated Management of Fruit Flies	Jesús Reyes
Madagascar	MAG5021	Implementing the Sterile Insect Technique (SIT) in Integrated Fruity Fly Control for High Quality Fruit Production	Rui Cardoso Pereira
Mauritius	MAR5019	Supporting a Feasibility Study Using the Sterile Insect Technique (SIT) for the Integrated Control of Mosquitoes	Jeremie Gilles
Mauritius	MAR5022	Reducing Insecticide Use and Losses to Melon Fly (<i>Bactrocera cucurbitae</i>) through Environment-Friendly Techniques to Increase Production in Different Areas, Phase II	Jorge Hendrichs Rui Cardoso Pereira

Morocco	MOR5032	Supporting Control of the Medfly Using the Sterile Insect Technique for Citrus Fruits and Early Fruits and Vegetables to Establish Low Medfly Prevalence Zones	Jesús Reyes
Oman	OMA5002	Assessing the Suitability of Sterile Insect Technique (SIT) and Related Techniques for Combating Date Palm Insect Pests	Marc Vreysen
Panama	PAN5020	Strengthening Technical Capacity to Control Mediterranean Fruit Fly Using the Sterile Insect Technique (SIT)	Jesús Reyes
Palau	PLW5001	Improving the Quality of the Fruits and Vegetables Through an Area-Wide Integrated Pest Management of <i>Bactrocera</i> Fruit Flies in Production Areas of Palau	Rui Cardoso Pereira
South Africa	SAF5013	Assessing the Sterile Insect Technique for Malaria Mosquitoes in a South African Setting	Jeremie Gilles
Senegal	SEN5033	Supporting the Operational Phase of Eliminating <i>Glossina palpalis gambiensis</i> from the Niayes Area by Promoting the Development of Integrated Stockbreeding	Marc Vreysen Andrew Parker
Seychelles	SEY5005	Enhancing the Melon Fruit Fly Area-Wide Integrated Pest Management Programme Using the Sterile Insect Technique to Improve National Food Security	Rui Cardoso Pereira
Sri Lanka	SRL5044	Supporting a Feasibility Study Using the Sterile Insect Technique (SIT) for Integrated Control of Mosquitoes	Jeremie Gilles
Sudan	SUD5034	Supporting a Feasibility Study on the Suitability of the Sterile Insect Technique as a Strategy for the Integrated Control of <i>Anopheles arabiensis</i>	Jeremie Gilles
Thailand	THA5052	Developing Sustainable Management of Fruit Flies Integrating Sterile Insect Technique with other Suppression Methods	Rui Cardoso Pereira
Uganda	UGA5036	Demonstrating the Feasibility of a Sterile Insect Technique Component as Part of an Area-Wide Integrated Pest Management Approach to Increase Livestock Productivity	Rafael Argiles
Vietnam	VIE5017	Supporting Area-Wide Integrated Pest Management to Improve the Quality of Fruit for Export	Rui Cardoso Pereira
Zimbabwe	ZIM5019	Improving Crop and Livestock Production through the Eradication of Bovine and Human Trypanosomosis in Matusadona National Park	Rafael Argiles

		Title Regional Projects	
Regional Africa	RAF5061	Supporting Capacity Building and a Feasibility Study on Control of Fruit Flies of Economic Significance in West Africa	Rui Cardoso Pereira
Regional Africa	RAF5062	Preventing the Introduction of Exotic Fruit Fly Species and Implementing the Control of Existing Species with the Sterile Insect Technique and Other Suppression Methods	Rui Cardoso Pereira
Regional Africa	RAF5065	Promoting the Sharing of Expertise and Physical Infrastructure for Mass-Rearing Mosquitoes and Integration of the Sterile Insect Technique (SIT) with Conventional Methods for Vector Control, among Countries of the Region.	Jeremie Gilles
Regional Africa	RAF5069	Supporting a Feasibility Study to Eradicate Tsetse from Southern Mozambique, South Africa and Swaziland	Marc Vreysen Rui Cardoso Pereira
Regional Africa	RAF5070	Supporting Area-Wide Tsetse and Trypanosomosis Management to Improve Livestock Productivity and Enable Sustainable Agriculture and Rural Development (Phase II)	Rafael Argiles
Regional Africa	RAF5072	Exploring the Use of Sterile Insect Technique as a Novel Technique for Control of Vector Mosquito for Chikungunya and Dengue (<i>Aedes albopictus</i>) in the Indian Ocean Region (PHASE I - 2014-2015)	Jeremie Gilles
Regional Asia	RAS5059	Supporting Area-Wide Integrated Pest Control of Native and Exotic Flies in the Middle East Subregion Incorporating the Sterile Insect Technique (SIT)	Jesús Reyes
Regional Asia	RAS5066	Promoting the Sharing of Expertise and Infrastructure for Dengue Vector Surveillance towards Integration of the Sterile Insect Technique with Conventional Control Methods among South and South East Asian Countries	Kostas Bourtzis Jeremie Gilles
Regional Asia	RAS5067	Integrating Sterile Insect Technique for Better Cost-Effectiveness of Area-Wide Fruit Fly Pest Management Programmes in South-east Asia	Rui Cardoso Pereira
Regional Europe	RER5020	Controlling Fruit Flies in the Balkans and the Eastern Mediterranean	Rui Cardoso Pereira
Regional Latin America	RLA5067	Supporting Capacity Building for Evaluation of Feasibility of a Progressive Control Programme for New World Screwworm	Jesús Reyes
		Title Interregional Project	
Interregional	INT5151	Sharing Knowledge on the Use of the Sterile Insect and Related Techniques for Integrated Area-Wide Management of Insect Pests	Jorge Hendrichs

Highlights of Technical Cooperation Projects

Strengthening the Planning, Design and Review of the Programme to Support the Implementation of Strategic Activities for Nuclear Technology and its Applications (RLA0052)

Coordination Meeting to Support Mediterranean Fruit Fly Eradication in Dominican Republic

Hispaniola has been a Mediterranean fruit fly-free island until some flies were detected in late 2014 in Punta Cana, a major tourist resort in the eastern Dominican Republic. This information was not made available immediately to regional and global plant protection organizations, and only a few months later, when the outbreak had expanded, were serious eradication efforts initiated. As a result, countries importing avocados, mangoes, peppers, tomatoes and other Dominican horticultural products closed their markets partially or completely, causing much hardship and major economic losses among producers and exporters.

The Guatemala/Mexico/USA Moscamed Programme, as well as FAO, IAEA, Inter-American Institute for Cooperation on Agriculture (IICA) and Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), are providing technical and financial support to the quarantine, surveillance, identification, data management, and control activities in the outbreak area. Additionally the country-wide trapping network is being strengthened, also for *Bactrocera* pest species. Current suppression efforts in the outbreak area include ground and aerial bait sprays and fruit destruction around detections. There has been good progress in establishing an emergency programme and in reducing the pest population, however, new satellite populations are being detected and the infested area has been expanding. Preparations have been initiated to integrate the sterile insect technique on an area-wide basis, so that suppressed populations can be eliminated and the establishment of new satellite populations avoided.

On May 19, a coordination meeting was held in Santo Domingo with the Minister of Agriculture and representatives of FAO, IAEA, IICA, OIRSA, USDA and the Moscamed Program. The objective was to avoid any duplication in the equipment, resources and other support being provided by the international organizations and to plan and coordinate future activities in order to increase the possibilities of succeeding in the eradication campaign. The on-going situation was analysed, the capacity and needs reviewed, and the support of each of the supporting organizations determined. A wider future goal will be to strengthen fruit fly surveillance and reaction capacities in other Caribbean countries.

Supporting Capacity Building for Evaluation of Feasibility of a Progressive Control Programme for New World Screwworm (RLA5067)

The First Meeting to Strengthen Surveillance and Response to Outbreaks of New World Screwworm (NWS) in Central America - Mexico under Project RLA5067 was held at the US-Panama Commission for the Eradication and Prevention of the Screwworm (COPEG) in Panama, 6-8 May 2015. The goal was to reach consensus on the development and implementation of a technical package of prevention and elimination of potential introductions of NWS into Central America and Mexico. The meeting was attended by participants from El Salvador, Honduras, Mexico, Nicaragua, Panama and the United States and representatives of the IAEA and the International Regional Organization for Plant and Animal Health (OIRSA).

As a result of this first coordination meeting it was agreed to develop and implement a technical package which includes a permanent preventive surveillance plan, a qualitative risk analysis that allows adjustment of the preventive plan, and an emergency protocol that addresses a critical route to remove a possible introduction of the NWS. This protocol should include periodic outbreak simulations to refine the operation of the plan.



Participants of the first meeting to strengthen surveillance and response to outbreaks of new world screwworm in Central America - Mexico.

The NWS is an important transboundary animal disease and significant zoonosis, which constrains sustainable livestock production, national/international trade and public health conditions. Although it has been eradicated, through the integrated use of the SIT, from the South of the United States, Mexico and Central American countries, possibilities of being re-introduced into the region are highly possible, so a preventive surveillance and response to an outbreak programme is critically needed. Currently Panama maintains an exclusion biological barrier along its border with Colombia by the use of a continuous sterile fly release programme.

Supporting the Operational Phase of Eliminating *Glossina palpalis gambiensis* from the Niayes Area (SEN5033)

Best Sustainable Development Practices on Food Security - Expo Milano 2015

Among 749 proposals admitted to the final evaluation process for Expo Milano 2015, the FAO/IAEA project "Eradication of the tsetse fly *Glossina palpalis gambiensis* from the Niayes in Senegal" was among 18 selected for "Best Sustainable Development Practices on Food Security".

The gold medal goes to Africa, with 7 winning projects, followed by Asia with 6, the Americas with 3 and Europe with 2. This is the geographic distribution of the 18 proposals selected for Expo Milano 2015 by an International Selection Committee (ISC) through an International Call that closed on 31 October 2014. The announcement was made by Prince Albert of Monaco, president of the ISC, and Maurizio Martina, the Italian Minister of Agriculture, Food and Forestry Policies, and Vice President of the ISC.

The Call is part of the "Feeding Knowledge" programme, which aims to raise awareness on the best scientific solutions in terms of food security and sustainable development.



Deployment of a monitoring trap to assess progress in the project "Eradication of the tsetse fly *Glossina palpalis gambiensis* from the Niayes in Senegal".

Of the 18 proposals selected, five projects will be shown in a film produced by Expo Milano 2015, while another 13 will be illustrated through photo stories. These film and photo stories will be seen by millions of visitors in Pavilion Zero, the largest thematic Pavilion in Expo history, developed in collaboration with the United Nations.

The Joint FAO/IAEA Division is proud to congratulate its counterpart, Baba M. Sall, Directorate of Veterinary Services of Senegal, and the various national and international collaborators, on the selection of the proposal "Eradication of the tsetse fly *Glossina palpalis gambiensis* from the Niayes in Senegal".

Promoting the Sharing of Expertise and Infrastructure for Dengue Vector Surveillance towards Integration of the Sterile Insect Technique with Conventional Control Methods among South and South East Asian Countries (RAS5066)

The mid-term review meeting of this regional TC project was held at Mahidol University, Bangkok, Thailand from 9-13 February 2015. Sixteen participants from eight countries (China, Indonesia, Malaysia, Pakistan, Philippines, Singapore, Sri Lanka and Thailand) as well as four experts (Brazil, French Polynesia, Spain and Sweden) attended this meeting.



Participants of the mid-term review meeting of TC project RAS5066 during a field trip to potential sites for SIT releases against *Aedes aegypti*.

In South and South-East Asian countries, mosquito-borne diseases continue to be a major public health problem with more than 2 billion people at risk of contracting dengue fever. The control of mosquito populations has largely relied on insecticide-based approaches. However, due to the problems associated with the insecticides (impact on human health and the environment, as well as increased phenomena of insecticide resistance), novel and environment-friendly approaches are being considered for the population control of mosquito vector species, such as the SIT.

Project RAS 5066 aims to transfer this technology to FAO and IAEA Member States. In the frame of the meeting, the activities of the project carried out during 2014 were thoroughly reviewed, including studies on the biology and behaviour of the target species, collection of baseline entomological data, as well as training on colonization and mass-rearing of mosquito species, irradiation studies, transport and releases, monitoring and surveillance, and quality control at all stages of the SIT package. Emphasis was given on dissemination activities and local public engagement programmes. In addition, the work plan for 2015 was discussed and approved including fellowships, procurement and an upcoming training course in Singapore.

Supporting a Feasibility Study Using the Sterile Insect Technique (SIT) for the Integrated Control of Mosquitoes (MAR5019)

Assessment of sterile male competitiveness in semi-field settings in Mauritius

Under project MAR/5/019 semi-field cage competitiveness experiments are being supported in Mauritius. To optimise the success of the pilot release trial in Panchvati, a comparison of mating success between 1, 3, or 5 day old irradiated *Aedes albopictus* males when competing with untreated males of the same age was performed in semi-field cages at the new rearing facility in Curepipe (see Figure).

Due to the cool weather and greater rainfall experienced in this inland region of the island, cages were protected under shelters, whilst still exposing the mosquitoes to the elements. After a mating period of 48h females were collected, blood fed and the viability of eggs used to determine whether each had mated a fertile or sterile male, or both.

Results indicated a good performance of the sterile males, which were as competitive as the wild ones when released at age 3 or 5 days in a sterile : fertile male ratio of 1:1. The average fertility in the semi-field cages was lower when 1 day old males were released, suggesting a higher participation in the matings from the sterile males.



Semi-field cages set up for competitiveness tests in the yard of the Curepipe facility.

As expected, an increased release ratio reduced the overall fertility in the cages, though there was no significant difference between the 3 ratios (1:1, 3:1 or 5:1 sterile to fertile males) for 1 or 3 day old males. At the release ratio of 5:1:1, the overall fertility was reduced to 20%.

Considering these results, an increased resting period prior to sterile male release (3 or 5 days) as previously suggested to improve performance, does not seem to be necessary to improve competitiveness. The optimum release for population suppression in these conditions therefore seems to be a 3:1 sterile to wild release ratio of 1 day old males.

Supporting Area-Wide Tsetse and Trypanosomiasis Management to Improve Livestock Productivity and Enable Sustainable Agriculture and Rural Development (Phase II) (RAF5070)

Regional Training Course on Free Open Source Software for GIS and Data Management applied to tsetse and trypanosomiasis control programmes. French Edition

The French edition of this regional training course was held in Vienna, Austria, from the 19 to 30 January 2015. The course was organized jointly by African Union-Pan African Tsetse and Trypanosomiasis Eradication Campaign (AU-PATTEC), FAO and IAEA and was attended by a total of 15 participants from 10 Member States (Angola, Burkina Faso, Chad, Gabon, Ivory Coast, Mali, Niger, Republic of the Congo, Senegal and Togo).



Participants of the Regional Training Course on free open source software for GIS and data management applied to tsetse and trypanosomiasis control programmes.

The course addressed the following topics among others:

- Free open source basics (Quantum GIS), software installation
- GIS basics
- Managing spatial Data
- Advanced spatial operations
- FOSS database basics.
- Introduction to satellite imagery analysis
- GPS data import
- Map composer
- Harmonization: data management in PATTEC programmes
- ATLAS on tsetse and Animal African Trypanosomiasis distribution at the country level.

Throughout the training, theoretical lessons and practical hands-on sessions were combined. The DVD tutorial "Using Open Source GIS Techniques in Insect Pest Control Programmes" was distributed as supporting material.

At the end of the training, a visit to the FAO/IAEA Insect Pest Control Laboratory in Seibersdorf was conducted and the participants had the chance to get acquainted with the latest technology and equipment for rearing not only tsetse flies, but also other insects.

Supporting Control of the Medfly Using the Sterile Insect Technique for Citrus Fruits and Early Fruits and Vegetables to Establish Low Prevalence Zones (MOR5032)

Progress on the adoption of the SIT to control the Mediterranean fruit fly in Morocco

The majority (75%) of Moroccan citrus destined for export, mainly to Europe, is produced in the Souss Valley, southern Morocco. The country exports annually around 400,000 tons of citrus and there is potential to double or triple this amount. The Mediterranean fruit fly is the major pest of citrus and is mainly controlled using insecticide sprays.

During the last years, citrus producers, exporting agencies and the National Plant Protection Organization (ONSSA) from the Ministry of Agriculture carried out a pilot project to suppress the Mediterranean fruit fly in 5,000 ha of the Souss Valley using an AW-IPM approach with a Sterile Insect Technique component. The pilot project was carried out with FAO/IAEA technical assistance and each week 8 million sterile male Mediterranean fruit flies were released that were imported initially from Madeira in Portugal, and later from the mass-rearing facility in Valencia, Spain.



Field visit of participants of the local SIT Technical Steering Committee to the construction site of the Mediterranean fruit fly rearing facility in Agadir, Morocco. Left to right: Mr. Mazih Ahmed - entomologist R&D SIT project support (IVAI), Mrs. Malika Bounfour – SIT project adviser (ONSSA, Rabat), Mr. Mohamed Berriga – growers association representative, Mr. Carlos Caceres – SIT facility design and planning advisor (FAO/IAEA), Mr. El Yacoubi Mohamed – engineer facility construction supervisor (ORNVA-SM), Mrs. Zhor Dehbi – SIT operations Manager (ONSSA, Agadir) and Mr. Dliou Amhed – field operations manager (citrus industry).

The SIT pilot was successful and prompted the Government of Morocco to make an alliance with the fruit industry to build in 2016, again with FAO/IAEA technical assis-

tance, a Mediterranean fruit fly mass-rearing facility and a sterile male release center large enough to process sufficient sterile males to cover the entire Souss Valley (40,000 ha of citrus plus surroundings). FAO/IAEA has assisted with the design of a mass-rearing, holding and release facility with a capacity to produce 130 million sterile males / week in its first phase, and eventually 200 million / week.

In August 2014, an agreement to establish an area-wide SIT programme against Mediterranean fruit fly in the Agadir region was approved by the Minister of Agriculture of Morocco and signed between the ONNSA, the Regional Office of Agriculture in Souss Massa (ORNVA-SM) and the Citrus Producers Association (Maroc Citrus).

Supporting Feasibility Studies for Using Sterile Insect Techniques as part of Area-Wide Integrated Pest Management for Control of Tsetse Flies (*G. morsitans centralis*) (ANG5012)

The goal of this project in Angola is to assess the feasibility of integrating the SIT as part of an area-wide campaign to eradicate a population of *Glossina morsitans centralis* in the central belt of Malanje, Kuanza-Norte and Kuanza-Sul. The estimated size of the target area is more than 32 000 km², according to the most recent distribution maps dating back several decades (maps generated in the 1970s by Ford and Katongo). To conduct the entomological monitoring by means of classical trap deployment for the baseline data collection in such an enormous and, in some cases, remote area requires huge human and material resources.

An alternative monitoring device has been designed to reduce the work involved in the entomological monitoring. This device includes a blue sticky plastic board mounted on a metal frame at the back of a 4WD vehicle which is continuously been photographed at defined time intervals (see Figure).



Sticky plastic board mounted at the back of a 4WD vehicle to record tsetse detections in Angola.

Each picture is georeferenced and analyzed by software developed specifically to detect the fly catches and assign them their GPS coordinates.

Preliminary tests of the system in the field show promising results, although several adjustments to the software, frame and camera settings will be required to increase the accuracy under changing environmental conditions.

Developing Sustainable Management of Fruit Flies Integrating Sterile Insect Technique with other Suppression Methods (THA5052)

Technical and economic characteristics of mangosteen production areas and associated fruit fly species and infestation levels in Chantaburi Province, Thailand are well identified and there is an obvious economic benefit that can be achieved through the implementation of this project.



Mangosteen fruit groves in Chantaburi Province, Thailand.

Data on fruit fly populations and host status already exist. However, a better and more systematic approach to collect baseline data is needed. Identification of alternative hosts in a large radius of the surrounding areas, their geo-reference and inclusion in maps and their relative importance (through fruit sampling) contributing during different times of the year to the increase of the fruit fly populations in mangosteen production areas is recommended.

A pilot 1500 ha area for fruit fly suppression is under implementation integrating sanitation with bait sprays, male annihilation technique and eventually the release of sterile *B. dorsalis* flies when populations are already at a low level. However, these and other future projects need collaboration among the relevant Thai organizations to achieve a more effective suppression of fruit flies.

This will contribute to the increase of fruit quality, exports and the farmer's income, together with a reduction of insecticide use with the consequent environmental and consumer benefits.

Demonstrating the Feasibility of a Sterile Insect Technique Component as Part of an Area-Wide Integrated Pest Management Approach to Increase Livestock Productivity (UGA5036)

As part of its plans to eliminate the tsetse fly *Glossina fuscipes fuscipes* from the Lake Victoria shore line, the Government of Uganda is considering to import in the future *G. f. fuscipes* pupae from the large rearing facility in Ethiopia. To make a final decision on this, the project needs to determine if the colony strain, which originates from the Central African Republic, is compatible with the local strain of this species.

The project has been collecting wild females from the Buvuma Islands in Lake Victoria to obtain pupae, but they have experienced difficulty with maintaining the wild material in the insectary as flies will not feed using an artificial membrane feeding system and so feed them on live animals.

The project, therefore, requested assistance with determining the necessary conditions for inducing wild flies to feed on artificial membrane feeding system to eliminate the need for live animal feeding.

For the flies to feed they must locate a suitable host, orient to and land on it, determine that the host is suitable, probe the skin and engorge on the blood. Location of the "host" is obviously not an issue as the caged flies are placed directly on the warm membrane. Previous publications from the 1980s and 1990s showed that various components of sweat are important in inducing colony flies to probe and that ATP and its analogues induce engorging, but these have not been tried singly or together on wild flies.

A mission was therefore sent to the National Livestock Resource Research Institute, Tororo, Uganda, to attempt to induce feeding in wild flies by combining these factors. The most potent probing stimulus reported previously was phenylalanine, so this was applied to the top surface of the membrane at the concentration typically found in human sweat (0.2 mM) and ATP was added to the blood at a rate of 1 mM.

Without ATP, phenylalanine induced probing in a proportion of the flies, but the flies failed to engorge. Limited feeding was, however, detected as faeces were observed on the tray under the cages exposed to phenylalanine alone or in combination with sodium chloride. When ATP was added to the blood, several flies were observed to fully engorge. The concentration and presentation of the stimulate needs to be optimized, but for the first time in Uganda wild flies can be maintained in the absence of live hosts, which will permit the conduct of the necessary compatibility tests.

Supporting a Feasibility Study on the Suitability of the Sterile Insect Technique as a Strategy for the Integrated Control of *Anopheles arabiensis* (SUD5034)

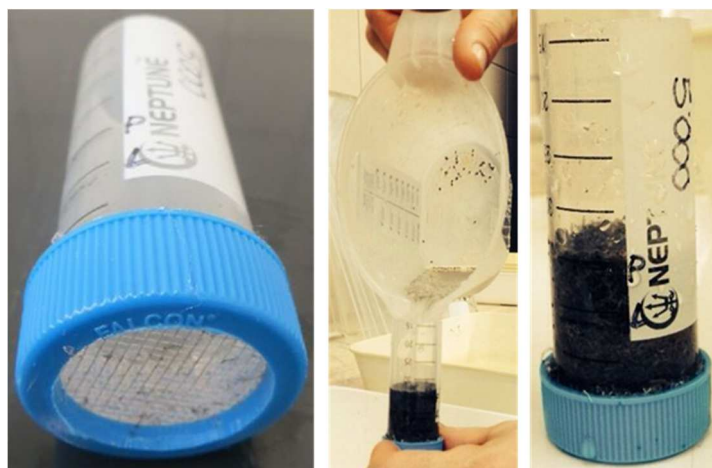
Guidelines for standardised mass-rearing of *Anopheles* mosquitoes

Working with an expert supported by project SUD5034, guidelines have been prepared on the methods that have been developed and optimised at the FAO/IAEA Insect Pest Control Laboratory for the rearing of *Anopheles* malaria vectors (see Figure).

Detailed descriptions and illustrative photographs along with short, easy to refer protocols in the guideline, offer a good basis for training existing and new staff hired to work in the mass-rearing facility in Khartoum, Sudan, whose construction is scheduled to start shortly.

Also beyond this direct application, this document will be very valuable in transferring the technology to Member States planning to rear *Anopheles* mosquitoes for control programmes with an SIT component, and can be provided alongside the mass-rearing equipment to help with their

use. Background information is also provided surrounding the biology of the mosquito, and how this was taken into account in designing the mass-rearing procedures, so that those using the guidelines will gain a good understanding of how to both carry out routine rearing tasks and how to monitor the colonies for problems and find solutions as they arise.



An example taken from the guidelines, explaining how to quantify pupae. Left to right: The modified 50 ml conical centrifuge tube; pupae being poured into the modified tubes; estimating the number of total pupae collected.

Coordinated Research Projects (CRPs) and Research Coordination Meetings (RCMs)

Project Number	Ongoing CRPs	Scientific Secretary
D4.10.23	Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade (2010-2015)	Jorge Hendrichs
D4.10.24	Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application (2012-2017)	Carlos Cáceres
D4.20.15	Enhancing Vector Refractoriness to Trypanosome Infection (2013-2018)	Adly Abd Alla Andrew Parker
D4.40.01	Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes (2013-2018)	Jeremie Gilles Kostas Bourtzis
D4.10.25	Dormancy Management to Enable Mass-rearing and Increase Efficacy of Sterile Insects and Natural Enemies (2014-2019)	Rui Cardoso Pereira
Project Number	New CRPs	Scientific Secretary
D4.20.16	Comparing Rearing Efficiency and Competitiveness of Sterile Male Strains Produced by Genetic, Transgenic or Symbiont-based Technologies (2015-2020)	Kostas Bourtzis
D4.40.02	Mosquito Handling, Transport, Release and Male Trapping Methods (2015-2020)	Rafael Argiles Jeremie Gilles
D4.10.26	Improved Field Performance of Sterile Male Lepidoptera to Ensure Success in SIT Programmes (2016-2021)	Marc Vreysen

Second RCM on *Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes*. 9–13 March 2015, Juazeiro, Brazil

This Second Research Coordination Meeting of the FAO/IAEA Coordinated Research Project on “Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes” was hosted by Jair Virginio and Margareth Capurro, Moscamed Facility, Juazeiro, Brazil and was attended by 15 scientists from Brazil, Cameroon, France, French Polynesia, Germany, Italy, Pakistan, South Africa, Spain, Sri Lanka and United Kingdom. In addition, eleven observers attended this meeting.

Twenty six progress report papers and related scientific presentations on irradiation and classical, molecular, and mechanical, behavioural and developmental approaches for sex separation were presented by participants and reviewed.



Participants of the Second Research Coordination Meeting of the FAO/IAEA Coordinated Research Project “Exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes” in front of the Moscamed Facility in Juazeiro, Brazil.

During the discussion it was concluded that the CRP has been very productive, with a much better understanding and exploitation of available classical genetic, molecular as well as mechanical, behavioural and developmental tools.

These can accelerate the production of robust and efficient sexing strategies for female elimination or can enhance existing methods towards male-only releases for SIT against mosquito vector species. In addition, all attendees underlined the valuable genetic information becoming available through the sequencing and annotation of the genomes of several mosquito disease vectors, as well as the recent progress on genome editing approaches. This knowledge can be exploited for the development and/or improvement of sexing strategies for the control of major mosquito vector species.

Consultants Meeting on Developing Sterile Mosquito Transport and Aerial Release Methods. 8–12 December 2014, Vienna, Austria

The experts that participated in the meeting advised on the need to develop and adapt as much as possible the existing equipment available for the handling, transport and release of other insect pests to the specific conditions for the mosquitoes. In this regard, adult mosquitoes are fragile insects that may require the development of special equipment and procedures to avoid mechanical injuries and allowing at the same time the work at large scale.

In addition, in case of smaller sizes of the targeted project areas, in particular for suppression in urban areas, and in view of the extremely light weight of the adult mosquitoes, alternative release methods such as unmanned aerial systems (UAS) should be considered.

Further research is required to assess the best stage for mosquito transport and release, to develop optimal environmental conditions during transport, to develop procedures and cages to hold the males, collect and pack the adults for the releases in the emergence facility and release them minimising the mechanical damage and optimising their effective distribution in the field.

Consultants Meeting on Mosquito Male Trapping Methods to Monitor the Efficacy of SIT Programme in the Field. 16–20 February 2015, Vienna, Austria

The SIT package that is being developed by the FAO/IAEA for *Aedes* and *Anopheles* mosquitoes, includes equipment and standard operating procedures for mass rearing, stage- and sex-separation and irradiation of males, to support the development of SIT programmes in several Member States.

Progress is also being made on the development of a functional release system adapted to the specific requirements of a mosquito suppression programme with an SIT component, with suitable methods for handling and transporting chilled, irradiated male pupae and adults prior to release.

It is essential for the efficient implementation of a release programme that the male mosquito population in the target area is monitored, both in the design phase of the programme to determine the scale of release required, during releases to tailor releases to real-time population data, and to assess the impact of the programme on the vector population. Most mosquito trapping and surveillance technology is aimed at the female vector, whereas the ability to monitor the sterile and wild male populations is essential for the SIT.

This technical meeting was therefore convened to advise the Member States of the regional Africa project (RAF5072) on the methods and trapping technologies available for male mosquitoes, what is still needed in order to achieve the level of monitoring needed for an AW-IPM programme with an SIT component, and to suggest possible companies or individuals who could develop prototype traps which could be tested in the field.

The consultants were selected from research groups with expertise in mosquito population surveillance, and commercial developers with experience in designing and developing devices similar to those which will be needed to trap male mosquitoes, and technologies which may be useful for future developments.

Existing trapping methods were considered for their efficacy for male trapping, and suitable tools for the monitoring of male mosquito population during SIT programmes identified, along with a discussion of considerations and suggestions for how to design and conduct surveillance programmes. Where new technology or techniques are required, the consultants discussed and recommended valuable avenues for further research, identifying partners who may be suitable to help develop traps or methodologies to meet those needs.

The requirements of a surveillance programme, divided into different operational phases, were detailed in the meeting report. In order to develop improved surveillance programmes for both *Aedes* and *Anopheles* species the following list of technical advancements were considered by participants to be priorities for development:

1. Efficient male and female traps
2. UAV methodology of swarm monitoring
3. Better mark-release recapture modalities
4. Independent monitoring tools – automated surveillance and real time remote data collection
5. Means for quality control and validation of trap effectiveness

Further research required to inform the design process include a better understanding of the male and female biology and ecology as related to attractive cues and behavioural studies in the laboratory and field, along with development of validation and modelling tools and protocols for community engagement.

Consultants Meeting on Improved Field Performance of Sterile Moths to Enhance SIT Application. 13–17 April 2015, Vienna, Austria

A consultants meeting was organized to review the status of Lepidoptera SIT with a focus on field performance of sterile moths. Many Lepidoptera pests require control to avoid significant losses in many cropping systems worldwide. Some major lepidopteran pests are also undergoing geographical range expansion. The SIT has been used successfully against a number of moth species, but despite successes, the wider development and deployment of SIT requires further investigation.

Previous coordinated research projects (CRP) on moths have addressed a number of constraints. Despite excellent progress by a previous CRP, there are a number of practical areas that limit expansion of SIT for Lepidoptera. Quality can be badly degraded during different processes related to the mass-rearing and release of sterile insects. These processes would be just as important for programs using other sources of sterility than irradiation, from transgenic, RNAi or other novel approaches under development.

The participants of the consultants meeting suggested a new CRP to address processes which need to be improved in relation to field performance of the sterile insects such as: improved rearing and maintenance of colonies based on selection for favourable behaviours, better collection and irradiation methods to enhance results, the application of two-sex or male-only release strategies, improved handling, transport and release methods, practical and effective methods for quality assessment, and better deployment strategies to improve cost-effectiveness and outcomes.

This objective of such a new CRP are to:

1. Determine the impact of different rearing parameters and behavioural traits that have an impact on competitiveness of sterile moths, by correlation of laboratory, semi-field and open-field performance
2. Determine the impact of adult and pupal collection and irradiation methods on field competitiveness
3. Determine the effect of sterile females on population suppression
4. Determine best practice methods of handling, transporting and releasing sterile moths to maintain field competitiveness
5. Determine the relative effectiveness of different methods for quality assessment and performance of sterile and wild moths
6. Develop best practice deployment of sterile insects in relation to hotspots, taking into account moth competitiveness and field performance.

Final RCM on Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade. 1–5 June 2015, Saint Pierre, France.

The final RCM was successfully held with 32 participants from 17 countries. The local organization and hospitality by Helene Delatte of CIRAD is gratefully acknowledged. All objectives of the meeting were accomplished, concluding a very fruitful CRP in which over 60 researchers from 22 countries were involved at different times over the last six years and four RCMs.

All the CRP outputs were achieved beyond expectations. One important outcome, based on considerably genetical, cytogenetical, chemical, morphometrical and behavioural evidence collected across Africa and the Asia/Pacific region, is the synonymization of the major pest species *Bactrocera papayae*, *B. philippinensis* and *B. invadens* with *B. dorsalis*. The fact that these four species are in reality only one species, the Oriental fruit fly, has very significant implications, simplifying SIT application and facilitating international agricultural trade. These changes have already been widely accepted by governments around the world.

For the *Anastrepha fraterculus* in Latin America it has been shown that this species is in reality a complex of eight separate species of different phylogenetic origin. Also for the *Ceratitidis* FAR pest complex in Africa it was determined that it consists of five fruit fly species. *Zeugodacus cucurbitae* on the other hand is a clearly one species throughout its distribution.

The CRP has resulted in many other outputs and outcomes, and produced ca. 100 scientific publications. The final results of the CRP are being edited for publication in a special issue of the peer-reviewed journal *ZooKeys*.



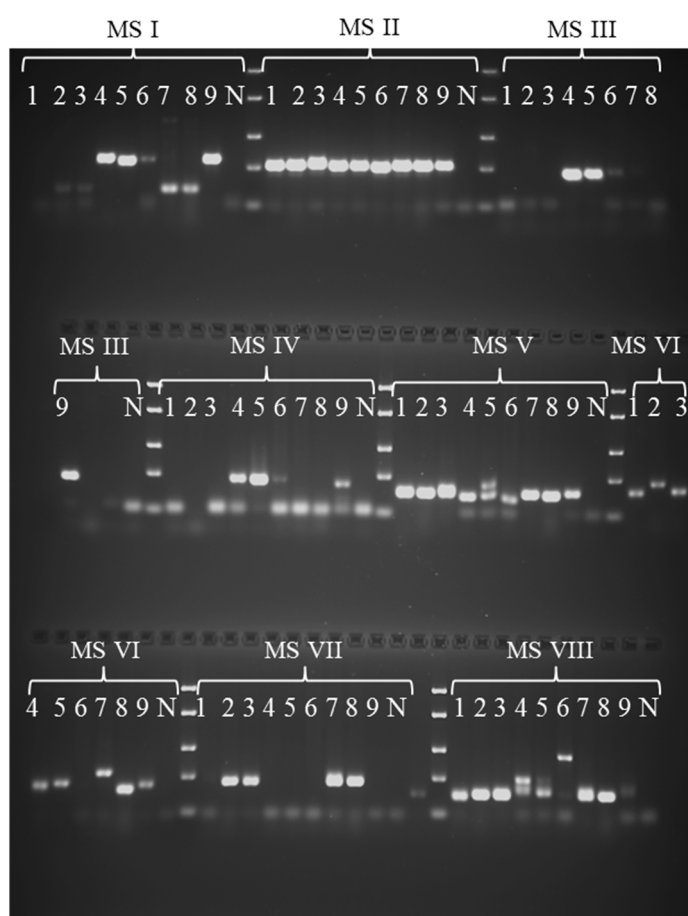
Participants of the final Research Coordination Meeting of the FAO/IAEA Coordination Research Project "Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade" Saint Pierre, La Réunion, France.

Developments at the Insect Pest Control Laboratory

INSECT GENETICS AND MOLECULAR BIOLOGY

Evaluation of different molecular markers for the identification of *Glossina* species

The correct species identification is of paramount importance for SIT applications and any other biologically-based pest control programme. At the Insect Pest Control Laboratory (IPCL), different tools are being exploited to develop a robust, easily applied, quick and low cost approach for the identification of different tsetse species. In order to develop and evaluate such methods and tools it is necessary to have: (a) extended natural collections covering the whole geographical range of the species, if possible; (b) colonized populations of the different species that can be used as reference; (c) combination of different classes of markers (such as genetic, molecular tools, morphometrics and others) and (d) combination of different tools of a specific class. In the case of molecular/genetic tools it is ideal to use independent markers.



Microsatellites as a tool to discriminate tsetse species. MS: microsatellite; numbers indicated different tsetse species, N: negative PCR control.

For this purpose, different molecular tools are currently being used and evaluated, including: (a) sequencing of different mitochondrial markers (*COI*, *COII*, *NADH*, *16S rRNA*); (b) PCR and electrophoresis (plus sequencing where needed) of the nuclear ITS1 region; (c) PCR and electrophoresis (plus fragment analysis where needed) of different microsatellite markers (see Figure) and (d) *Wolbachia* infection status and genotyping, including possible chromosomal insertions, of the collections. This analysis could also be extended to additional symbionts.

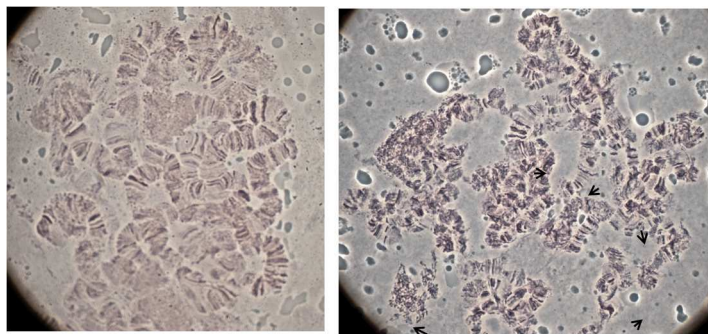
These tools are currently evaluated against: (a) old DNA collections made during the last two decades and still available at the IPCL; (b) new DNA collections established at the IPCL during the last months and (c) laboratory colonies either kept at the IPCL or available in other laboratories. The currently available results suggest that each molecular tool tested provides valuable information which allows the species identification with high accuracy and speed, either as stand-alone or in combination with others.

Cytogenetic studies to support resolution of species complexes in Tephritidae

A variety of mechanisms can lead to speciation phenomena in insects. Chromosomal rearrangements play a catalytic role in insect speciation. Symbiotic bacteria, particularly the ones interfering with reproduction and mating behaviour seem to be another key speciation factor. Recognizing the importance of correct species identification for SIT applications and related pest control programs, particularly as concerns species complexes, the IPCL and collaborators (A. Zacharopoulou and A. Avgustinos from University of Patras, Greece; P. Mavragani and E. Drosopoulou from Aristotle University of Thessaloniki, Greece and J. Cladera, S. Lanzavecchia and C. Giardini from Instituto Nacional de Tecnología Agropecuaria, Argentina) have initiated a thorough cytogenetic and symbiotic analysis in members of the *Bactrocera dorsalis* species complex, *Anastrepha fraterculus* species complex and *Ceratitis FAR* complex.

During the last months, the construction of the polytene maps of *A. fraterculus* (Tucuman morphotype) as well as of a member of the *Ceratitis FAR* complex was initiated and it is near to completion. Once available, these maps will provide a very useful tool for comparative cytogenetic analysis between different members of the respective complexes. Cytogenetic analysis is also very useful to identify hybrids produced from crosses between closely or distantly related members of a complex. Here we present two images from such a cytogenetic analysis (see Figure) indicating the characteristic asynapses of the homologous chromosomes which are present in F₁ hybrids from crosses between *B. dorsalis* s.s. and a distantly related species, but absent in

the F_1 hybrids of similar crosses with a closely related species.



Cytogenetic analysis of F_1 hybrids among *B. dorsalis* s.s. and a closely related member of the complex (left). Note the absence of extended asynapses among the homologous chromosomes. Cytogenetic analysis of F_1 hybrids among *B. dorsalis* s.s. and a distantly related member of the complex (right). Note the presence of the characteristic extended asynapses of the homologous chromosomes indicated by arrows.

PLANT PESTS

Medfly VIENNA-8 genetic sexing strain gut symbionts and their use as probiotic supplements in larval diet

As mentioned in a previous newsletter (IPC NL 82), the IPCL has been using culture-dependent and culture-independent approaches to characterize and exploit the gut-associated microbiota of key insect pests and disease vectors that might be potential targets for the SIT. We have been using three different microbiological media and isolated at least three different bacterial species from guts dissected from larvae as well as adults (males and females) of different age. Using *16S rRNA* gene-based sequencing analysis, it was shown that two out of the three isolated bacterial species belong to Enterobacteriaceae, which are commonly present in the guts of tephritids. One of these species was cultured in large quantities and used as a probiotic supplement in the larval diet of the Mediterranean fruit fly VIENNA 8 genetic sexing strain. Their effects on different biological parameters were assessed, including immature survival and development, mating competitiveness, survival under food and starvation and flight ability. Preliminary results suggest that addition of gut symbiotic bacteria as probiotic supplement in the larval diet has significant effects on developmental parameters, particularly if the bacteria are provided alive, which is currently under investigation. We expect to inform our readers about the final results of this study in one of the future issues.

Phytosanitary treatment research

Recent research under the USDA/FAO/IAEA collaborative agreement, "Development of phytosanitary treatments for exotic tephritid fruit flies" has been conducted to determine the most cold-tolerant stage of fruit flies, to assess if the host affects cold treatment efficacy, and also to compare whether populations of the same species differ in cold and

heat tolerance. The IPCL has approximately 10 populations of *Bactrocera dorsalis*, a similar number from the *Anastrepha fraterculus* complex, and multiple populations of other key species of economic importance for this testing. The results of this research guide phytosanitary treatment scheduling at the national and international levels among Member States of FAO and IAEA, as well as the International Plant Protection Convention. Research on cold treatment doses for phytosanitary control of *Anastrepha grandis*, *Bactrocera occipitalis*, and *Zeugodacus tau* has been initiated; cold treatments are currently not available for use on hosts of these species.

LIVESTOCK PESTS

Impact of irradiation on tsetse symbionts

Tsetse flies harbour three symbiont bacteria *Wigglesworthia glossinidia*, *Sodalis glossinidius* and *Wolbachia pipientis* and a pathogenic virus (salivary gland hypertrophy virus (SGHV)). The SIT has proven to be an effective method to eradicate tsetse fly populations.

To minimize any risk that released sterile male tsetse flies might contribute in endemic sleeping sickness areas to disease incidence before achieving eradication, they are fed trypanocide-treated blood. However, the development of strategies to produce tsetse strains that are refractory to trypanosome infection is also desirable. One approach is to modify *Sodalis* to produce anti-trypanosome factor(s) in the sterile males that are destined for release. Therefore, the impact of irradiation on *Sodalis* is being analysed by a visiting scientist from Turkey, Güler Demirbas, and primary results indicate that irradiating tsetse male flies did not increase the mutation rate (SNPs) in the *Sodalis* genome. The irradiation, however, showed a negative impact on the multiplication of *Sodalis* and SGHV as compared with non-irradiated flies. In contrast, irradiation increased the prevalence of *Wolbachia* as compared with non-irradiated flies. Further investigation to analyse the impact of irradiation on tsetse symbionts is in progress.



Zelda Moran sexing pupae of *Glossina palpalis gambiensis* using the new near infrared tsetse pupal sorter.

Sorting of tsetse pupae by sex

Work on pupal sorting using a near infrared seed sorter has continued in the laboratory with some success in separating male from and female pupae, but the setup of the seed sorter still needs to be optimized. Nevertheless, the apparent success of the system to sort pupae led us to consider what exactly is detected by the machine. Using near infrared LEDs as controlled light sources and a simple silicone sensor camera, with the infrared filter removed, we have been observing pupae under a low power microscope. Surprisingly the puparium, which in visible light is dark brown to almost black, under near infrared light is almost transparent, permitting direct observation of the developing pupa within. Development of male and female pupae has been observed throughout the complete pupal period and features characteristic of the stage of development noted.

HUMAN DISEASE VECTORS

Towards aerial release methods for mosquitoes

There are many aspects which make up a successful SIT programme. One area which is crucial is the development of adequate handling, transport and release methods. Mosquitoes are much less robust than some other types of insect, for which previous SIT projects have been successful, thus handling and transporting them will require great care. In order to limit mortality during transport, the sterile male mosquitoes could be chilled to immobilize them prior to being packaged, as well as for the entire duration of the transport.

The distance from a mass-rearing facility to a release site will vary depending on each SIT programme as will the temperature at which flight is disabled in each mosquito species. It is therefore necessary to conduct a series of preliminary experiments encompassing various species, temperatures and durations in order to assess the resulting impact upon survival and performance.

In support of the new CRP on mosquito transport, release and male trapping methods, several preliminary experiments have been initiated to answer some of the fundamental questions outlined above. To narrow down the range of temperatures which may be suitable for transport, *Anopheles arabiensis*, *Aedes aegypti* and *Ae. albopictus* were filmed inside a climate chamber across a wide temperature range using Go Pro cameras to capture how quickly flight was disabled at each tested temperature. Additionally, after removal from the chamber, the time mosquitoes required to resume flight was recorded. These preliminary data enabled us to select a narrow range of temperatures at which to test the impact of the cold temperatures for several periods on mosquito survival.



Mosquito survival was followed for 14 days post-cold exposure to determine the optimum temperature at which they should be transported whilst immobilised.

Initial results indicate that in both *Aedes* species, survival on day 14 after cold-exposure remained above 80%. It was noted that survival in *Ae. aegypti* was slightly higher when compared to *Ae. albopictus*. *An. arabiensis*, which in comparison to *Aedes* are much less robust also fared well with survival remaining above 70% for all 4 tested temperatures at 1, 4 or 8 hours exposure. Exposure for 24 hours resulted in survival ranging between 60 and 70% across the experimental temperature range. When taking into consideration natural mortality, these results are very encouraging and pave the way for more extensive testing to be carried out in order to handle, transport and release sterile male mosquitoes successfully.

Characterization of the gut-associated microbiota of mosquito species

Studies in fruit flies have clearly shown the importance of gut-associated microbiota on the life history traits of the host species. Using culture-dependent and culture-independent approaches, we have initiated the characterization and exploitation of the gut-associated bacterial of mosquito species that are potential targets for the SIT. Recently we have focused on *Ae. albopictus* (GUA strain) using three different microbiological media to characterize cultivable bacterial species associated with different developmental stages of this mosquito: 3rd or 4th instar larvae, 1 day old males and females (teneral), 14 day old males and 14 day old females fed only with sugar or blood. Eggs were also included in the study. The same samples will be also analysed with *16S rRNA* gene-based next generation sequencing approaches. The effect of irradiation treatment on the structure of bacterial communities and density levels of different bacterial species will also be assessed. The ultimate goal is to identify key bacteria which could be used as probiotics to improve the mass-rearing of *Ae. albopictus* as well as to mitigate any negative effects irradiation might have on the mating competitiveness of sterile males.

Reports

Expert Consultation on Phytosanitary Treatments in *Bactrocera dorsalis* Complex, 1–5 December 2014, Okinawa, Japan

The Expert Consultation was initially called by the International Plant Protection Convention to address the issue of *Bactrocera invadens* invading Africa and the lack of phytosanitary treatments against that supposed new species. However, before the meeting the multiauthor publication by Schutze et al. 2015 was issued that finally concluded that *B. invadens* was, in fact, *B. dorsalis*. The meeting was hosted by the Plant Protection Division of the Japanese Ministry of Agriculture, Forestry and Fisheries in Okinawa, Japan during 1-5 December 2014. The objectives of the meeting were to provide a forum for researchers to share data and discuss the issues related to phytosanitary treatments against *B. dorsalis* and related species, identify currently scheduled treatments, and develop a harmonized research protocol for developing additional treatments.

The recent synonymization of *B. invadens*, *B. papayae* and *B. philippinensis* with *B. dorsalis* was reviewed and its implications for phytosanitation in Africa, which are mainly:

1. Areas already infested by *B. dorsalis* not under regulatory control cannot quarantine against its host commodities in Africa
2. Treatments approved, considered, or researched against *B. dorsalis* may now be considered for use in Africa
3. The host range for *B. dorsalis* in Africa is now expanded over what it was for *B. invadens*.

The history of the development of phytosanitary treatments against *B. dorsalis* was critically reviewed going back to early studies with vapour heat and irradiation almost 90 years ago. Challenges identified include lack of voucher specimens, insufficient characterization of measurement of efficacy, use of artificial infestation without testing possible effect on efficacy, excessive infestation rates, lack of determination of most tolerant stage, and excessive mortality in control groups. In summary, it was noted that differences continue to prevail between the commercial environment and the research supporting phytosanitary treatments and that researchers must be mindful that the assumptions made in their work do not reduce treatment efficacy. Researchers ought to make as few assumptions as possible, stated or not, and explain and justify those that are made. Priorities for research were identified:

1. Compare artificial with natural infestation for effects of the former on efficacy
2. Determine if host cultivar or species affects efficacy
3. Evaluate effect of rate of temperature change on cold and heat treatments.

Regarding the future, the group decided to incorporate efforts to develop more phytosanitary treatments against *B.*

dorsalis into the newly formed Phytosanitary Temperature Treatment Expert Group and jointly write a peer reviewed paper, Phytosanitary Treatments against *Bactrocera dorsalis*: Current Situation and Future Prospects.

Meeting of BINGO (Breeding Invertebrates for Next Generation BioControl Training Network) 22-23 January 2015, Wageningen, The Netherlands

Regulations for the use of pesticides are getting stricter to ensure food safety and protect ecosystems. Population control of agricultural pests by using biologically-based control approaches, including the SIT and/or natural enemies, has great potential to deal with these two demands.

BINGO, an EU-funded Innovative Training Network in the frame of Marie Skłodowska-Curie Actions, aims to advance current knowledge in biocontrol practice by simultaneously training 13 early stage researchers (to be recruited as PhD students) in an extensive suite of interdisciplinary skills. The kick-off meeting was attended by 16 participants from different institutions, universities, research centers and international organizations from 8 countries.



Participants of the kick-off meeting of the Second Research Coordination Meeting of BINGO (Breeding Invertebrates for Next Generation BioControl Training Network) at the Wageningen University, Wageningen, Netherlands.

The PhD project of the ESR who will be based at the FAO/IAEA Insect Pest Control Laboratory in Seibersdorf, Austria, will focus on testing whether symbiotic bacteria play an important role in the nutrition and the fitness of both the olive fly *Bactrocera oleae* and its parasitoid *Psytalia lounsburyi*. The objective is to determine, characterize and exploit the symbiotic flora in both the olive fly and the parasitoid for optimizing the mass-rearing of *B. oleae* and its parasitoid towards AW-IPM applications with an SIT component. Such applications require efficient, high-quality and cost-effective mass-rearing of both olive fly and its parasitoid.

Seventh European Mosquito Control Association (EMCA) Workshop. 23-26 February 2015, Valencia, Spain

The 7th EMCA Workshop was attended by over 150 researchers, technicians and manufacturers involved in mosquito control. During the meeting research papers were presented and discussed in seven scientific sessions on subjects including discoveries in control techniques, new bio-icides, mosquito surveillance and disease transmission. Two special symposia were included namely 'Mosquito management in protected wetlands and nature conservation' and 'National strategies in mosquito control'.

The significance of invasive *Aedes* species and the diseases they carry was universally accepted by members of EMCA, and many talks were on the subject of tracking the spread of *Aedes albopictus* and *Ae. aegypti* in different countries. Whilst Bti has clearly been the dominant method of mosquito control, there is some openness to the need for alternative and complementary tools, and some enthusiasm for testing the SIT. Presentations were given on the work that the FAO/IAEA Insect Pest Control Laboratory is doing to develop the 'mosquito SIT package', and on the remaining research questions, as well as the potential SIT programmes under planning against *Aedes sticticus* in Sweden and *Aedes* species in Thailand.

Ninth Session of the Commission on Phytosanitary Measures (CPM), International Plant Protection Convention, FAO. 16-20 March 2015, Rome, Italy

The FAO Deputy Director General Ms. Helena Semedo, welcomed the CPM members to FAO and stressed the need to increase efforts to protect food security and environment, to ensure trade is safe from pests of plants and that a failure to avoid the spread of plant pests and diseases could have disastrous consequences on agriculture production and food security for millions of poor farmers.

The CPM adopted:

- Annex 3 of International Standards for Phytosanitary Measures (ISPM) 26 on "Phytosanitary procedures for fruit fly (Tephritidae) management"
- Annexes of ISPM 28 on
 - "Cold treatment for *Bactrocera tryoni* on *Citrus sinensis*"
 - "Cold treatment for *Bactrocera tryoni* on *Citrus reticulata* x *C. sinensis*"
 - "Cold treatment for *Bactrocera tryoni* on *Citrus limon*"
 - "Irradiation for *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor*"

The draft ISPM on "Determination of host status of fruit to fruit fly (Tephritidae)" was the subject of lengthy discussions on the definition of "non-natural host vs conditional host vs semi-natural host". As a result, the draft ISPM was returned to the IPPC Standard Committee for further discussion. Additionally, a side event organized by the IPPC Secretariat with support of the Joint FAO/IAEA Division took place with the participation of more than 75 delegates. At the event, the results of an expert consultation on phytosanitary treatments for the *Bactrocera dorsalis* complex held in December 2014 in Japan were presented.

Second International Workshop on *Aedes albopictus*, the Dengue Vector. 23-26 March 2015, Guangzhou, China

This workshop on *Aedes albopictus* was held to facilitate interactions between the relatively small research community working with this aggressive and invasive mosquito vector of viral diseases, and to maintain communication and co-operation between a group established at the first meeting. Research was presented on the genomic sequencing, vectorial capacity, diapause behavior, odorant responses, microbiota and means of surveying and controlling this species, with a focus on the Chinese situation and strains. Presentations were given on the control of *Aedes albopictus* populations using SIT and *Wolbachia* based approaches.

Consultants Meeting on Generic Approach for the Development of Genetic Sexing Strains for SIT Applications. 1-5 June 2015, Vienna, Austria

The use of genetic sexing strains (GSS) can greatly improve the cost-effectiveness and the efficacy of sterile insect technique applications for agricultural pests. However, GSS are a prerequisite for the mosquito SIT applications since females are responsible for the transmission of human pathogens. A generic approach is therefore necessary for the development of genetic sexing strains for SIT applications.

A Consultants Meeting on "Generic Approach for the Development of Genetic Sexing Strains for SIT Applications" was organized in Vienna (Austria). Five consultants and three observers attended this meeting. The participants reviewed the current knowledge available in this field and identified the advantages and the disadvantages of the currently existing methods including those of the emerging technologies. In addition, they provided recommendations on the necessary future research and development steps that need to be undertaken in that field. This included specific recommendations on how the Insect Pest Control Laboratory could contribute to the achievement of this important task, which would greatly facilitate the development and application of the SIT package for different insect agricultural pests and disease vectors.

Announcements

Call for Submission of Research Proposals for a new FAO/IAEA Coordinated Research Project on *Improved Field Performance of Sterile Moths to Enhance the Application of SIT*

Lepidoptera are key pests that require control to avoid significant losses in many cropping systems worldwide. The sterile insect technique (SIT) has been used successfully against a number of moth species, but despite successes, the wider development and deployment of SIT has important issues that need further investigation. Optimal quality of the released sterile males is a prerequisite for success in SIT programs, and insect quality can be badly degraded during many processes involved with the mass-rearing and release of sterile insects.

A new CRP will be implemented to address important processes that need to be improved and tied to field performance of the insects. These would include:

- improved rearing and maintenance of colonies based on selection for favourable behaviours
- better collection and irradiation methods to enhance results
- the application of two-sex or male-only release strategies
- improved handling, transport and release methods
- practical and effective methods for quality assessment, and
- better deployment strategies to improve cost-effectiveness and outcomes.

This objective will be met by:

- Determining the impact of different rearing parameters and behavioural traits that have an impact on competitiveness of sterile moths, by correlation of laboratory and semi-field and open-field performance
- Determining the impact of adult and pupal collection and irradiation methods on field competitiveness
- Determining the effect of sterile females on population suppression
- Determining best practice methods of handling, transporting and releasing sterile moths to maintain field competitiveness
- Determining the relative effectiveness of different methods for quality assessment and performance of sterile and wild moths
- Developing best practice deployment of sterile insects in relation to hotspots, taking into account moth competitiveness and field performance.

The expected duration of the CRP is 5 years (2016-2021) and the first Research Coordination Meeting is planned for **4-8 July 2016 in Durban, South Africa**.

Scientists and researchers who are interested in collaborating in this new CRP should contact Marc Vreysen (m.vreysen@iaea.org). Information on the IAEA Coordinated Research Programme and how to apply for research contracts and research agreements can be found at <http://www-crp.iaea.org/>. Applications should be submitted by **30 November 2015** to Official.Mail@iaea.org.

Announcement of FAO/IAEA Regional Training Courses

- Regional Training Course on *The Use of Population Genetics and GIS to Identify Isolated Tsetse Populations* (under Regional TC Project RAF5070). 21 September–2 October 2015, Addis Ababa, Ethiopia. **(Deadline for nominations: 20 July 2015).**
- Regional Training Course on *Taxonomy and Identification of Fruit Fly Pest Species for Southeast Asia* (under Regional TC Project RAS5067). 16–20 November 2015, Brisbane, Australia. **(Deadline for nominations: 31 August 2015).**

Application procedure: Nominations should be submitted on the standard IAEA application form for training courses/workshops (downloadable from: <http://www-tc.iaea.org/tcweb/participation/astraine/default.asp>).

Completed forms should be endorsed by and submitted through the official channels established (either the Ministry of Foreign Affairs, the National Atomic Energy Authority, the Office of the United Nations Development Programme, the Office of the FAO Resident Representative or the Ministry of Agriculture).

The completed forms must be submitted to the International Atomic Energy Agency, Vienna International Centre, P.O. Box 100, 1400 Vienna, Austria. Advance nominations by facsimile (+43-1-26007) or email (official.mail@iaea.org) are welcome.

In Memoriam

Peter Teal (1953-2015)

It is with great sorrow that I announce the passing of Peter Teal, Research Leader of the Chemistry Research Unit at the Center for Medical, Agricultural and Veterinary Entomology (CMAVE), USDA-ARS Gainesville, Florida. Peter had been suffering from pneumonia.



Peter Teal assumed the role of Research Leader of the Chemistry Research Unit in 2003 and served in that position until 2015. In 2014, he also began service as Acting Research Leader of the Subtropical Horticulture Research Station, Miami, Florida.

Peter Teal was a Supervisory Re-

search Insect Physiologist, and he obtained both his B.Sc. and M.S. Degrees from the University of Ottawa and his Ph.D. in Entomology from the University of Florida. Dr. Teal began his career with ARS at CMAVE in 1990. Prior to that, he was an Assistant Professor at the University of Guelph from 1983 to 1986, and an Associate Professor at the University of Florida (1986 to 1990).

He was an outstanding scientist with an international reputation and had achieved the level of super grade. His research focused on isolation and identification of naturally produced compounds that affect the behavior and reproduction of insects and in developing control and monitoring strategies for control of invertebrate pests. He received many awards recognizing his research and leadership. In 1991, he received the USDA Outstanding Early Career Scientist Award. Later, Dr. Teal was recipient of the Arthur S. Flemming Award for Science (2002) and Researcher of the Year Award from the Florida Beekeeping Association (2005).

Peter Teal was active in technology transfer efforts related to protecting plants and honey bees from devastating pests while protecting the environment. He worked closely with industry using CRADAs and MTAs such as developing a new attractant for small hive beetles, a significant pest of honeybees. In 2014, he received the South Atlantic Area Senior Research Scientist of the Year "For excellence in chemical ecology research and advancing the surveillance and control of agricultural insect pests." His research has been documented in over 200 scientific publications and patents.

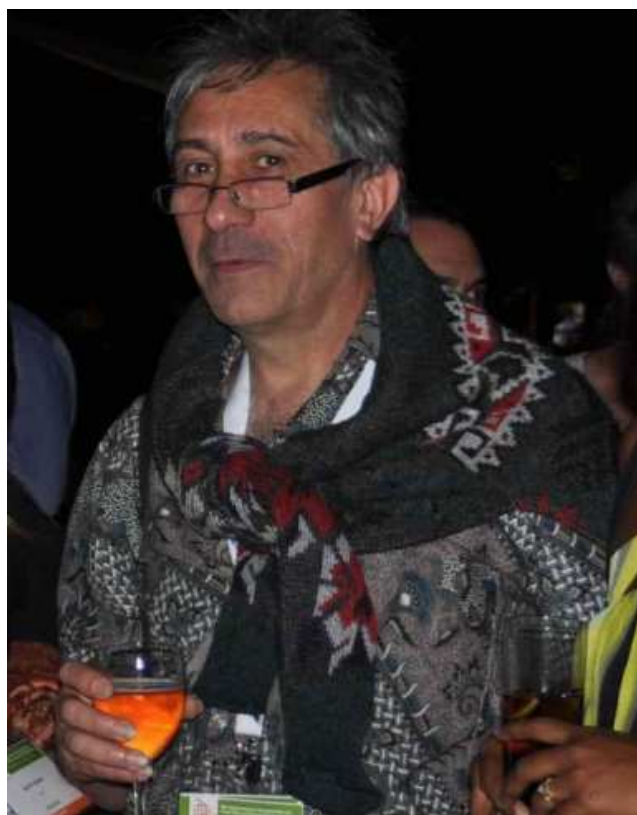
This is a great loss for USDA, ARS, the agricultural industry, and all of the fellow employees and friends who worked closely with Peter. We will all miss Peter's humor, compassion, guidance, and excellence in science and leadership. Our thoughts and prayers are with his wife Kathy and son Evan.

Source: Deborah Brennan, Director, Southeast Area, ARS, USDA

Serge Quilici (1955-2015)

This weekend we received the sad news that Serge Quilici died after a long illness. Serge was a senior researcher at CIRAD in La Reunion and well known in the world of fruit fly researchers. His research activities and interests were very broad and included the fields of fruit fly behavior, invasive species, interspecific competition, host selection, semiochemicals and the role of plant volatiles in attracting female flies among others.

He coordinated several international projects and supervised numerous students, and most people will be familiar with his extensive work and that of his many collaborators. He was also one of the founders of TEAM and member of the Steering Committee in the first years of its existence.



But first and foremost he was a very kind person, always willing to assist you in any way he could. Being around with him was always fun and I am sure many of you will have pleasant memories of the evenings together at one congress or another (as the picture below, taken by Massi Virgilio during the TEAM Symposium, Valencia 2010).

Source: Marc De Meyer, TEAM Steering Committee

After the deep sadness in which Serge Quilici's death left us, we would like to greatly thank you for all your kind messages and thoughts you sent. All your messages were compiled and handed to his family.

If you want to post the same or another thought for Serge's memory, a webpage has been opened on the website of our research unit (<http://umr-pvbm.cirad.fr/actualites/serge-quilici>).

Source: Hélène Delatte, CIRAD, La Réunion, France

Other News

Okanagan - Kootenay Sterile Insect Release Program Nominated for International Award

The Okanagan-Kootenay Sterile Insect Release (OKSIR) Program in British Columbia, Canada was nominated for a prestigious Integrated Pest Management Achievement Award, awarded at the 8th International IPM Symposium in Salt Lake City, Utah, March 23-26. The OKSIR program was nominated by the National Pesticide Risk Reduction Program, Agriculture and Agri-food Canada, Ottawa.

The OKSIR program has been successfully controlling codling moths in the apple and pear growing areas of the Okanagan and Similkameen areas for the last 20+ years. The program has been garnering a lot of international interest from areas and companies looking to recreate its success with codling moths (France, New Zealand, USA), false codling moth (South Africa), or looking to use its approaches with other pests (Switzerland, France).



IPM Achievement Award winners at 8th International IPM Symposium in Salt Lake City, Utah, March 23-26, 2015.

The program operates from Salmon Arm to Osoyoos, and into the Similkameen Valley, and is led by the General Manager, Cara Nelson. "It's an honour to be formally recognized by the international integrated pest management community," she states. "Our whole valley can be proud of the OKSIR program, not just for the amount of pesticides kept out of the environment because the program is in place, but also for the support it provides for our fruit growers, who are important to our local economy and our Okanagan way of life."

Since the beginning of the program, the amount of pesticides used against codling moth has been reduced by 96%. Many orchardists in the valley have not had to spray their trees for codling moth for the last 15 years. If a chemical spray is required, most growers need only use a single

well-timed application, instead of the three or more they needed prior to the program's implementation.

Fred Steele, president of the British Columbia Fruit Grower's Association said about the OKSIR program in his letter of support for the nomination, "The Okanagan-Kootenay Sterile Insect Release program is an asset for both our pome fruit growers and the residents of the valleys that live near and amongst the orchards." He also noted, "The success of the program has led to other local fruit industries, including cherry and grape growers, expressing interest in applying the area-wide approach to their own pests." OKSIR staff spend the spring and summer releasing sterile codling moths in apple and pear orchards. These sterile moths attract and mate with wild moths. Because the released moths have been sterilized, they can't produce healthy eggs, the eggs can't hatch, so the fruit is not damaged, and the moth population doesn't increase.

The Achievement Award winner was announced in February 2015. Cara Nelson, General Manager for OKSIR, gave a presentation at the International IPM Symposium as a part of a session organized by the Organisation for Economic Co-operation and Development (OECD). The session focused on the role of partnerships and stakeholder collaboration in the success of region-wide IPM programs.

Source: Cara Nelson, OKSIR (www.oksir.org , 3 February 2015)

The SIT programme against False Codling Moth, in South Africa. The sterile moths are dropped by means of gyrocopters

In 1976, the insect *Thaumetotibia leucotreta* , also known as False Codling Moth (FCM) established itself in Citrusdal, South Africa's oldest citrus exporting region. This put citrus exports in a precarious position, with some growers reporting losses of up to 50-60% of their crops.

XSIT, an initiative of Citrus Research International (CRI) to market the Sterile Insect Technique (SIT), helps in reducing the spread of FCM and reduces the populations. Nevill Boersma, Quality and Technical Manager of XSIT, explains that "FCM larvae enters the fruit, which consequently drops and rots. In the past, growers used lots of chemicals and pesticides with mixed success, but in 2006, with FAO/IAEA support leading to successful SIT trials conducted by Hendrik Hofmeyr and his team, the X-STERILE-INSECT-TECHNIQUE, or XSIT, was first commercialised."

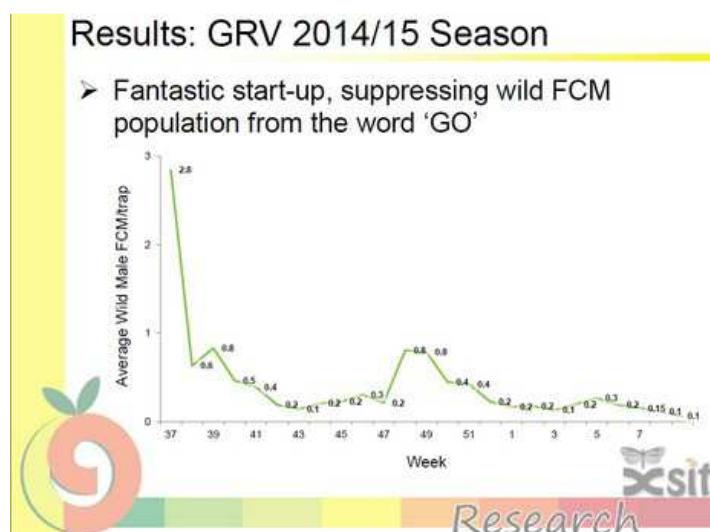
XSIT currently has 12,700 hectares contracted, both in the Eastern and Western Cape provinces to control FCM. "We produce 5 million sterile moths every day, which outnumber wild moths in the treated areas by at least 10 to 1. If the latter try to mate with the former, there will be no offspring

and as a result, the population continues to drop every year,” explains Boersma.

The institution also elaborates statistical data. “For every 5 hectares, we have one data station; a trap with sticky pads and a pheromone. We can then count how many males have been caught and see what the ratio is between wild and sterile and where the hotspots are. This is done 365 days a year,” according to Boersma.

To ensure their spread, the sterile moths are dropped on the contracted orchards by means of gyrocopters. Their efficiency is guaranteed, as the institution makes use of a computer statistical/mathematical program and GPS. Once released, the sterile moths will start mating with any wild moths around, thus ensuring zero offspring.

To illustrate the programme’s effectiveness, Nevill shares the success story of Gamtoos River Valley (GRV), which was initiated in 2014 (see graph). “In this area, 2.8 males were being caught on average per trap every week, and within a week we managed to bring it down to less than one wild male per trap. These growers have no need to spray chemicals anymore and can safely export the fruit to the European markets.”



It is worth stressing that, while FCM is not the only problematic insect on citrus, it entails a great phytosanitary risk, since the pest is not found anywhere else in the world. “Naturally, Europe and America want to keep it out, and with South Africa as the world’s second largest citrus exporter, the FCM can pose a huge threat, but with these suppression data (in conjunction with a post-harvest treatment), the risk is considerably reduced.”

The only problem, according to Nevill, is convincing some growers about its installation, as unlike similar programmes in other countries, in South Africa it is not subsidised by the Government and therefore involves a considerable extra cost to the grower.

Some packing houses have actually started to demand the implementation of XSIT. “For the export market, they must be able to show data. With two strikes detected by the De-

partment of Agriculture, they must stop exporting, and if an interception takes place in Europe, the farm responsible will be liable for the costs,” affirms Nevill.

In conclusion, Nevill stresses that, “most of the growers are supportive, as they are paying for a really good service, and if there are any problems, they can get in touch with us and we will solve them in the same day.”

Source: www.freshplaza.com (28 April, 2015)

Moscamed Brazil: Ten years of efficiency in management, technology and innovation!

Ten years ago, the São Francisco Valley has gained a strong ally in the fight against the fruit fly, *Ceratitis capitata*, the pest of economic importance that causes damage to the regional fruit production. Born on 11 April, 2005 the Biofactory Moscamed Brazil, aims to produce and release sterile males, through the use of the Sterile Insect Technique (SIT).

Set in the north of Bahia, in the city of Juazeiro, the first biofactory of Brazil is well known as an excellence facility in applying the best techniques, conducting training, research and development, as well as events that promote the diffusion of technical and scientific information. It was the entity chosen by the IAEA to validate the technology of x-rays to sterilize insects.

Over ten years, many significant partnerships were established with public and private entities, national and international, to better serve agriculture and human health. Currently, it is recognized by the Ministry of Agriculture and Livestock (MAPA) and the Government of Bahia as a social profit organization. It also has the recognition of public utility by the Juazeiro City Hall and is also recognized as a research entity by the National Scientific and Technological Development Council (CNPq).

Becoming a world reference in the application of new technologies attracted the partnership with the University of São Paulo (USP) to develop an innovative method for the biological control of *Aedes aegypti* - the *Aedes Transgenic Project*, which aims at population reduction of this dengue and chikungunya vector, by means of a lethal gene that prevents the generation of descendants.



The feeling that surrounds us is that of gratitude, for we know that in these ten years of history we have had the collaboration of professionals and partners engaged in achieving the common good, making possible the prompt service

to our customers. It was a time of learning and maturation, which showed us that the work is just beginning.

Undoubtedly, our greatest gift is the friendships established, national and global recognition of our work, respect for nature, exercise of our values and meeting our day-to-day our mission.

Source: *Moscamed Brazil* (www.moscamed.org.br)

A set of multi-entry identification keys for African frugivorous flies

Tephritid fruit flies or "true" fruit flies include approximately 500 genera and 4800 valid species, whose vast majority is represented by phytophagous species. Among them, frugivorous flies represent approximately 30% of all tephritid species and occur in tropical and temperate regions of all continents except the Antarctic. In Africa, damage on commercial fruits and crops is caused mainly by polyphagous species belonging to the genera *Bactrocera*, *Ceratitis*, and *Dacus*. Other genera with are *Capparimyia*, *Carpophthoromyia*, *Neoceratitis*, *Perilampus* and *Trirhithrum*.

The morphological identification of African frugivorous flies largely depends on the use of classical dichotomous keys. These keys are available for most African genera, with the important exception of the genus *Ceratitis*, whose species can only be identified through separate subgeneric keys. The main disadvantage of single-entry keys is that species identification inevitably fails whenever the user is not able to select any of the dichotomous character states listed in the key.

For these reasons, the Royal Museum for Central Africa (RMCA), in collaboration with the FAO/IAEA and the Belgian Directorate-General for Development Cooperation developed an electronic multi-entry identification key for African frugivorous flies. The keys provide a professional identification tool that is also accessible to non-specialised dipterists. This work was largely based on datasets that were created by Marc De Meyer and Ian White within the framework of past taxonomic revisions. Character scores for more than 68.000 entries were transferred into seven separate data sets, imported into the LUCID platform (www.lucidcentral.org) and used as the main data sources to build seven identification keys (including a total of 394 taxa) for species identification within genus or genus group. Additionally, a "pre-key" for genus designation was built ex novo by selecting a set of 23 characters that were deemed to be informative for generic separation.

To facilitate identification, characters were grouped into head, thorax, wings, legs and abdomen character sets. The user has the possibility of following a three steps identification procedure that considers characters of straightforward

use at first, followed by characters of more and more difficult interpretation. This aims at facilitating identification and reducing the risk of misidentification. Identification can also be restricted to species of economical importance to speed up the ID of more commonly trapped / intercepted taxa. The keys include thousands of images clearly illustrating name and position of each character on the insect body as well as showing how the same character state looks in different species.

Once a tentative identification is obtained (or when the list of candidate species is reduced to a few taxa), the keys give the possibility of verifying the correspondence between the examined voucher and (1) the species description as it appears in the published scientific literature and (2) images from the RMCA and from the London Natural History Museum tephritid collections. Discrepancies between the examined voucher and available images (as it might result from the occurrence of multiple character states for a species) can then be verified through hyperlinks to the species description. Information regarding the taxonomic status, geographic distribution and collection specimens of each taxon is also available through hyperlinks to Encyclopedia of Life and to the Belgian Biodiversity Platform (section of the Global Biodiversity Information Facility). Links to the Barcoding of Life Database website allow verifying occurrence and geographical coverage of DNA barcodes available.

Reduced versions of the keys (i.e. without images) are provided as downloadable supplementary files to the Virgilio et al. 2014 paper in ZooKeys. Full version can be freely accessed online (<http://keys.lucidcentral.org/keys/v3/fruit-flies/>) or directly requested to the authors: (massimiliano.virgilio@africamuseum.be; marc.de.meyer@africamuseum.be).

The keys are regularly updated and used during the training organised or co-organised by RMCA with FAO/IAEA). Last updates included morphological characters for the separation of genera *Bactrocera* and *Zeugodacus* and for identification of *Ceratitis rosa* genotypes R1 and R2.

Source: Massimiliano Virgilio & Marc de Meyer (Royal Museum for Central Africa, Tervuren, Belgium).

Economic Species of Dacine Fruit Flies in the Pacific Islands

A poster (see next page) was recently produced by Luc Leblanc from the Department of Plant and Environmental Protection Sciences, University of Hawaii. The poster can be visited and downloaded at:

<http://www.herbarium.hawaii.edu/fruitfly/pdf/Pacific%20FF%20Poster%201.2.pdf>.

Tsetse fly: can castration end one of Africa's oldest development problems?

Radiation castration is helping to eradicate tsetse populations that have been preventing farmers from using animals to work their land.

From the Sahara to the Kalahari, the tsetse fly has plagued African farmers for centuries. Dating back to prehistoric times, this tiny insect – just eight to 17 mm long – has prevented farmers from using domestic animals to work the land, limiting production, yields and income. The economic impact of the tsetse fly on Africa has been estimated to be as much as \$4.5bn. But a simple dose of radiation castration is helping to eradicate the pests in small pockets, enabling farmers to bring animals back into agriculture.

When tsetse flies bite, the parasites (trypanosomes) transferred cause sleeping sickness in humans, and nagana (African animal trypanosomosis) in animals – mostly cows, horses, donkeys and pigs. The parasites cause confusion, sensory disturbances and poor coordination in humans, and fever, weakness and anemia in animals. Both can be fatal if left untreated.

“In areas with tsetse, people tend not to use intensive forms of agriculture where you use animals or manure on the fields,” says Marcella Alsan, assistant professor of medicine at Stanford University who has researched the tsetse fly’s impact on development. Farmers in these areas use slash and burn agriculture instead but “the issue with that strategy is that you can’t constantly use the land in the production cycle, so it supports fewer people,” says Alsan. Until now, insecticides have been used to prevent animal trypanosomosis but this will not eradicate the fly; it reduces numbers but they always come back. Infected animals are treated with trypanocides but these drugs can be expensive, parasites are increasingly developing resistance to them, and in some cases up to 70% of trypanocides sold in Africa are counterfeit or fake.

But now scientists are hoping that a small dose of sterilisation could contribute to a more permanent and environmentally-friendly end to Africa’s oldest development problem. The procedure is unusual and fairly slow to work. Wild tsetse flies are captured from a target population and used to mass-rear pupae in factories. The male pupae are separated from the females, put into canisters, and subjected to doses of radiation to make them sterile. The sterilised males are then released by plane (roughly once a week, 100 flies per sq km) to mate with wild females. As a female tsetse fly only mates a few times in her life – once in most cases – any sexual activity with a sterilised male means that female will never produce any offspring. It’s a slow process of birth control, reducing tsetse fly numbers generation by generation.

The upside is that, unlike pesticides, flies cannot develop resistance to this procedure – the sterile insect technique

(SIT) – and eradication is sustainable as whole tsetse populations are permanently eliminated. The downside is that the process is slow, expensive and can currently only be used against isolated tsetse populations.

Sterilising insects is a growing industry. The technique is also used against various species of fruit fly, moth, and screwworm fly and there are mass-rearing facilities for these insects around the world, producing hundreds of millions of sterile males per week.

Sterile tsetse flies, however, are so far only produced on a much smaller scale in Burkina Faso, Ethiopia and Tanzania. “This is the major bottleneck to rapidly expanding the use of the SIT on a larger scale against some selected tsetse populations,” says Jorge Hendrichs, head of insect pest control for a programme run by the Food and Agriculture Organisation (FAO) and the International Atomic Energy Agency (IAEA). “Due to the slow reproduction rate of tsetse females it takes a big colony to produce a relatively small output of sterile males.”

On Zanzibar, the tsetse population of *Glossina austeni* was eradicated by the SIT in 1997, with a major positive socio-economic impact. On Unguja Island, part of the Zanzibar archipelago, the number of small farmers holding indigenous cattle increased from 31% in 1985 to 94% in 2002, and the average income in farming households increased by 30% from 1999 to 2002.

In the Niayes region of Senegal an isolated tsetse population of *Glossina palpalis gambiensis* is now being successfully targeted but the SIT cannot, unfortunately, get rid of these flies across Africa once and for all.

“Major tsetse eradication campaigns [integrating various methods including the SIT] would stretch over many decades and would require billions,” says Hendrichs. “At the moment, such funding is simply not available. A campaign would require the backing of major international donors to approach the funding levels necessary to begin thinking about such an undertaking.”

In addition, says Hendrichs, total eradication of tsetse is not actually needed as only six or seven of the 32 known species and subspecies attack livestock, and aerial spraying of insecticides can eradicate populations in areas of sparse vegetation.

But for those areas where tsetse populations can be eradicated the benefits are huge. Farmers wouldn’t need to shell out for expensive insecticides or trypanocides and they would finally be able to adopt modern husbandry methods and use animals in the fields to increase crop production. “They would also be able to create small agro-industries – making things like milk and yoghurt to sell for an extra source of income,” says Hendrichs. “This of course leads to higher production output and more money for their families.”

Source: *The Guardian* (18 February, 2015).

Relevant Published Articles

Participation of irradiated *Anopheles arabiensis* males in swarms following field release in Sudan

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Abstract

BACKGROUND: The success of the SIT depends on the release of large numbers of sterile males, which are able to compete for mates with the wild male population within the target area. The processes of colonisation, mass production and irradiation may reduce the competitiveness of sterile males through genetic selection, loss of natural traits and somatic damage. In this context, the capacity of released sterile *Anopheles arabiensis* males to survive, disperse and participate in swarms occurring at varying distances from the release site was studied using mark-release-recapture techniques.

METHODS: In order to assess their participation in swarms, irradiated and marked laboratory-reared male mosquitoes were released 50, 100 or 200 m from the known site of a large swarm on three consecutive nights. Males were collected from this large swarm on subsequent nights. Over the three days a total of 8,100 males were released. Mean distance travelled (MDT), daily probability of survival and estimated population size were calculated from the recapture data. An effect of male age at the time of release on these parameters was observed.

RESULTS: Five per cent of the males released over three days were recaptured. In two-, three- and four-day-old males, MDT was 118, 178 and 170 m, and the daily survival probability 0.95, 0.90 and 0.75, respectively. From the recapture data on the first day following each release, the Lincoln index gives an estimation of 32,546 males in the natural population.

DISCUSSION: Sterile *An. arabiensis* males released into the field were able to find and participate in existing swarms, and possibly even initiate swarms. The survival probability decreased with the age of male on release but the swarm participation and the distance travelled by older males seemed higher than for younger males. The inclusion of a pre-release period may thus be beneficial to male competitiveness and increase the attractiveness of adult sexing techniques, such as blood spiking.

The full paper was published in: *Malaria Journal* 2014, 13:484.

Polyandry in the medfly - shifts in paternity mediated by sperm stratification and mixing

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Abstract

BACKGROUND: In the Mediterranean fruit fly (medfly), *Ceratitis capitata*, a highly invasive agricultural pest species, polyandry, associated with sperm precedence, is a recurrent behaviour in the wild. The absence of tools for the unambiguous discrimination between competing sperm from different males in the complex female reproductive tract has strongly limited the understanding of mechanisms controlling sperm dynamics and use.

RESULTS: Here we use transgenic medfly lines expressing green or red fluorescent proteins in the spermatozoa, which can be easily observed and unambiguously differentiated within the female fertilization chamber. In twice-mated females, one day after the second mating, sperm from the first male appeared to be homogeneously distributed all over the distal portion of each alveolus within the fertilization chamber, whereas sperm from the second male were clearly concentrated in the central portion of each alveolus. This distinct stratified sperm distribution was not maintained over time, as green and red sperm appeared homogeneously mixed seven days after the second mating. This dynamic sperm storage pattern is mirrored by the paternal contribution in the progeny of twice-mated females.

CONCLUSIONS: Polyandrous medfly females, unlike *Drosophila*, conserve sperm from two different mates to fertilize their eggs. From an evolutionary point of view, the storage of sperm in a stratified pattern by medfly females may initially favour the fresher ejaculate from the second male. However, as the second male's sperm gradually becomes depleted, the sperm from the first male becomes increasingly available for fertilization. The accumulation of sperm from different males will increase the overall genetic variability of the offspring and will ultimately affect the effective population size. From an applicative point of view, the dynamics of sperm storage and their temporal use by a polyandrous female may have an impact on the Sterile Insect Technique (SIT). Indeed, even if the female's last mate is sterile, an increasing proportion of sperm from a previous mating with a fertile male may contribute to sire viable progeny.

The full paper was published in: *BMC Genetics* 2014, 15(Suppl 2):S11.

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