

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf

Insect & Pest Control Newsletter

No. 77

July 2011

http://www-naweb.iaea.org/nafa/ipc/index.html http://www.fao.org/ag/portal/age-index.html

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ISSN 1011-274X

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AEA

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Symbiotic bacteria of insects play significant role in the physiology, nutrition, development, reproduction, speciation and ecology of their hosts. One of the best-studied insect symbionts is Wolbachia which is able to manipulate the reproductive properties of its insect hosts by inducing phenomena such as parthenogenesis, feminization, male-killing and cytoplasmic incompatibility, which is a kind of male sterility. These images show Wolbachia-infected Drosophila testes (A), spermatids (B), ovaries (C and D), whole embryo (E) and embryo at the syncytial stage (F). Wolbachia bacteria are shown in yellow-green while Drosophila DNA is shown in red (Image courtesy: Zoe Veneti and Kostas Bourtzis). There is an increasing interest in the use of Wolbachia and other symbiotic infections in an applied context to control insect pests and disease vectors. A new Coordinated Research Project focused on the use of symbiotic bacteria in support of SIT will be initiated (see announcement inside).

To Our Readers

As reported in some of our previous newsletters, both FAO and IAEA have been undergoing considerable transformation as a result of a major on-going reform process of FAO that started in 2009 and which is scheduled to be fully implemented by 2013. In addition, the IAEA has seen a complete change of senior management and in January 2011 Mr Daud Mohamad was appointed Deputy Director General Nuclear Sciences and Applications and Head of the Department which includes the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Mr Mohamad holds a Bachelor of Science degree from Universiti Kebangsaan in Malaysia, a Master of Science degree from McMaster University in Canada, and a PhD in High Level Radioactive Waste Management from the University of Glasgow/Scottish Universities Research Reactor Centre in the UK. He has published more than 70 technical papers and was Chief Editor for the book entitled 'Nuclear Science and Technology'. Prior to joining IAEA Director General Amano's senior management team, Mr Mohamad held the position of Director General of the Malaysian Nuclear Agency from September 2004. He had joined Nuclear Malaysia in 1978 and was one of the pioneer staff of the organization. Mr Mohamad is married to Prof. Dr. Siti Rahayah Ariffin. We welcome him and wish him much success in his new position.



The IAEA has been implementing AIPS, a new IAEA wide Information System for Programme Support, representing a drastic transformation of processes. Until recently there were over 60 different and independent internal information systems and AIPS is replacing most of them with one Oracle product. AIPS also entails the adoption of IPSAS, the International Public Sector Accounting Standards, which is used in a majority of international organizations, involving independentlymaintained standards for financial reporting, considered best practice for organizations like ours. AIPS is being introduced in stages or 'plateaus'. The first plateau is devoted to Finance, Procurement, Transportation and the operational parts of Programme & Project Management. This went live in January 2011, in tandem with our adoption of IPSAS. Plateau 2 is scheduled for 2012. Unfortunately, the introduction of AIPS has over the last months caused a major disruption of functioning procedures, in the procurement of items and other routine internal processes, resulting in lengthy delays in payments of consultants, companies, research contracts, etc. Hopefully all this change has not disrupted our support to Member States too much. We apologize to those counterparts and colleagues who have already been affected. We expect that the situation will gradually improve, eventually enabling better information availability and improved programme management across the IAEA.

In terms of new publications, a special issue of Genetica on 'Molecular Technologies to Improve the Effectiveness of the Sterile Insect Technique' was recently published. The special issue, edited by Gerald Franz, represents the outcome of a five-year Coordinated Research Project managed and funded by the Joint FAO/IAEA Division. Genetic change can be introduced either using classical genetics or modern biotechnology, specifically genetic transformation. Both approaches have advantages and disadvantages related to stability and productivity under mass-rearing conditions, transferability of systems between pest species, regulatory approval, public acceptance, etc. The special issue contains 15 scientific papers from leading researchers in the field of classical and modern biotechnology and reviews the state-of-theart in the use of genetics and molecular biology to generate improved strains for the SIT application that produce only male insects for sterilization and release, and/or carry identifiable markers to disciminate released and wild insects in the field.



A second publication, 'Rearing Codling Moth for the Sterile Insect Technique' is a text book that was published under the FAO Plant Production and Protection Paper series. It was authored by Dr V.A. Dyck who is a former staff member of the Joint FAO/IAEA Division. Efficient and effective mass-rearing of the target insect is a fundamental component of the SIT, but its complexity for lepidopteran pests is very often under-estimated. This FAO/IAEA book compiles and summarizes all available information on the rearing of the codling moth in relation to the SIT, and is therefore a very valuable contribution in support of ongoing and future management programmes against lepidoptera pest insects. Aspects such as colonization, adult and larval diet, sexing, quality control, shipment, disease control, data recording and management are described. The integration of sterile insects within the context of area-wide integrated management of the codling moth offers great potential as shown by the on-going SIR Programme in Canada, which has succeeded for over a decade in drastically reducing insecticide applications in the Okanagan valley in British Columbia (see Other News).



I would also like to call your attention to a new six-year FAO/IAEA Coordinated Research Project on 'Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application' that was approved to start in 2012. The objective of the CRP is to extend previous efforts to improve sterile insect quality by manipulating

during mass-rearing and pre-release handling processes the diverse symbiotic microorganisms associated with fruit pests targeted in SIT operations, such as moth and fruit flies. These microorganisms will be applied in larval and adult diets as probiotics and their use explored as novel pest control tools with the aim to reduce production costs of the sterile insects and increase their quality. We are encouraging the submission of relevant research contract and agreement proposals.

Finally with respect to staffing, I would like to inform you that Andrew Jessup finished his assignment at the Insect Pest Control Laboratory in March and has moved back to his family and previous position in Australia. We very much appreciate the valuable contributions Andrew made to the sub-programme, especially with respect to the mating studies with members of the Bactrocera dorsalis and Anastrepha fraterculus complexes and the progress made with the development of olive fly massrearing techniques, a species for which the implementation of the SIT has been hampered for decades due to the lack of effective mass-rearing protocols. He was also the Scientific Secretary of the CRP on 'Development of Mass-Rearing for New World (Anastrepha) and Asian (Bactrocera) Fruit Fly Pests in Support of SIT', and participated in the Technical Panel on Phytosanitary Treatments of the International Plant Protection Convention. We will miss him as a friend and colleague and wish him much success in his future career with the New South Wales Department of Plant Industry.

> Jorge Hendrichs Head, Insect Pest Control Section July 2011

Insect Pest Control Subprogramme

http://www-naweb.iaea.org/nafa/ipc/index.html http://www.fao.org/ag/portal/age-index.html

Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture P.O. Box 100, 1400 Vienna, Austria Tel.: (+) 43 1 2600 21628; Fax: (+) 43 1 26007

Insect Pest Control Laboratory, FAO/IAEA Agriculture & Biotechnology Laboratories 2444 Seibersdorf, Austria Tel.: (+) 43 1 2600 28404; Fax: (+) 43 1 26007 2874

Staff of the Insect Pest Control Subprogramme

Name	Title	E-mail	Extension	Location
Jorge Hendrichs	Section Head	J.Hendrichs@iaea.org	21628	Vienna
Rui Cardoso Pereira	Entomologist (Plant Pests)	R.Cardoso-Pereira@iaea.org	26077	Vienna
Udo Feldmann	Entomologist (Tsetse/Screwworms)	U.Feldmann@iaea.org	21629	Vienna
Jesús Reyes	Entomologist (Plant Pests)	J.Reyes-Flores@iaea.org	26062	Vienna
Magali Evrard	Senior Secretary	M.Evrard@iaea.org	21633	Vienna
Adrene Despars	Team Assistant	A.Despars@iaea.org	21632	Vienna
Marc Vreysen	Laboratory Head	M.Vreysen@iaea.org	28404	Seibersdorf
Adly Abd Alla	Virologist (Tsetse)	A.Abdalla@iaea.org	28425	Seibersdorf
Gerald Franz	Geneticist (Plant Pests)	G.Franz@iaea.org	28419	Seibersdorf
Jeremie Gilles	Entomologist (Mosquitoes)	J.Gilles@iaea.org	28407	Seibersdorf
Andrew Parker	Entomologist (Tsetse)	A.Parker@iaea.org	28408	Seibersdorf
Tamara Wimberger	Team Assistant	T.Wimberger@iaea.org	28267	Seibersdorf

Forthcoming Events (2011-2012)

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

Third RCM of CRP on Biology of Male Mosquitoes in Relation to Genetic Control Programmes. 3-7 October 2011, Bologna, Italy.

Third RCM of CRP on Applying GIS and Population Genetics for Managing Livestock Insect Pests. 10-14 October 2011, Bobo Dioulasso, Burkina Faso.

Second RCM of CRP on Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade. 30 January-3 February 2012, Brisbane, Australia.

Final RCM of CRP on Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens. 26-30 March 2012, Vienna, Austria.

First 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. 21-25 May 2012, Vienna, Austria.

Third RCM of CRP on Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control. 22-26 August 2012, Phoenix, AZ, USA.

Third RCM of CRP on Development and Evaluation of Improved Strains of Insect Pests for SIT. 20-24 August 2012, Daegu, Republic of Korea.

II. Consultants and Expert Meetings

Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies under the International Plant Protection Convention, FAO. 22-26 August 2011, Recife, Brazil.

Consultants Meeting on Enhancing Vector Refractoriness to Trypanosome Infection. 3-7 October 2011, Vienna, Austria.

Consultants Meeting on Diapause Management to Facilitate the Rearing of Temperate/Overwintering Pests. 16-20 April 2012, Vienna, Austria.

Consultants Meeting on the Effects of Mosquito Production and Release Methods on Male Competitiveness. 8-12 October 2012, Vienna, Austria.

III. Other Meetings/Events

38th Congreso de Sociedad Colombiana de Entomologia (Socolen). 27-29 July 2011, Manizales, Colombia.

VII Curso Internacional de Capacitação em Moscas das Frutas de Importância Económica e Quarentenária, 11-19 August 2011, Juazeiro (Bahia) and Petrolina (Pernambuco), Brazil.

XXI Curso Internacional Sobre Moscas de la Fruta. 15-26 August 2011, Metapa de Dominguez, Chiapas, Mexico.

30th International Scientific Council for Trypanosomiasis Research and Control. 12-16 September 2011, Bamako, Mali.

FAO/IAEA Training Course on Taxonomía, Ecología y Control de Moscas de Importancia Económica (under TC Project RLA5057). 26-30 September 2011, Panama City, Panama.

International Symposium on Mass Production & Commercialization of Arthropod Biological Control Agents. 21-24 October 2011, Beijing, China.

Standards Committee Meeting, International Plant Protection Convention, FAO. 7-11 November 2011, Rome, Italy.

FAO/IAEA Workshop on Tsetse Wing Morphometrics (under TC Project RAF5059). 14 November - 2 December 2011, Pretoria, South Africa.

FAO/IAEA Regional Training Course on Standardized Entomological Monitoring, Data Collection and GIS-Aided Data Processing as Needed for Area-Wide Integrated Pest Management Campaigns Against the Tsetse and Trypanosomosis Problem (under TC Project RAF5060). 14 November - 2 December 2011, Bobo-Dioulasso, Burkina Faso.

FAO/IAEA Regional Training Course on Standardized Collection and Processing of Tsetse Flies for Molecular Tsetse Population Genetic and Morphometric Analyses (under TC Project RAF5060). 21 November - 5 December 2011, Muguga-Nairobi, Kenya.

Second International Meeting of Tephritid Workers of Europe, Africa and the Middle East. 2-6 July 2012, Kolymbari, Crete, Greece.

8th Meeting of the Working Group on Fruit Flies of the Western Hemisphere. 30 July – 3 August 2012, Panama City, Panama.

FAO/IAEA Workshop on Assessing Quality Management Aspects of Lepidoptera Mass-produced for the Sterile Insect Technique in a Large Operational Setting. 20-21 August 2012, Phoenix, AZ, USA.

Past Events (2010-2011)

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

Second RCM of CRP on Biology of Male Mosquitoes in Relation to Genetic Control Programmes. 1-5 February 2010, Vienna, Austria.

Second RCM of CRP on Applying GIS and Population Genetics for Managing Livestock Insect Pests. 22-26 February 2010, Bali, Indonesia.

Third RCM of CRP on Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens. 26-30 July 2010, Nairobi, Kenya.

First RCM of CRP on Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and International Trade. 2-6 August 2010, Vienna, Austria.

Second RCM of CRP on Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control. 15-19 November 2010, Stellenbosch, South Africa.

Final RCM of CRP on Development of Standardized Mass-Rearing Systems for Male *Anopheles arabiensis* Mosquitoes. 7-11 March 2011, St. Clotilde, La Réunion, France.

Second RCM of CRP on Development of Generic Irradiation Doses for Quarantine Treatments. 11-15 April 2011, College Station, TX, USA.

Second RCM of CRP on Development and Evaluation of Improved Strains of Insect Pests for SIT. 9-13 May 2011, Nanjing, China.

II. Consultants and Expert Meetings

Consultants Meeting on Development of Generic Design for Mosquito Mass-Rearing Facility. 3-7 May 2010, Vienna, Austria.

Consultants Meeting on Applications of Area-Wide IPM Programmes as Part of Systems Approaches for Pest Risk Management. 7-11 June 2010, Vienna, Austria.

Technical Panel on Phytosanitary Treatments under the International Plant Protection Convention, FAO. 26-30 July 2010, Tokyo, Japan.

Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies under the International Plant Protection Convention, FAO. 4-8 October 2010, Vienna, Austria.

Consultants Meeting to Update International FAO/IAEA/USDA Manual on Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies. 18-22 October 2010, Vienna, Austria.

Consultants Meeting on the Role of Bacteria on Nutritional Status and Reproductive Success in Fruit Fly Pests in Support of SIT Application. 7-11 February 2011, Vienna, Austria.

III. Other Meetings/Events

FAO/IAEA Regional Training Course on Surveillance of Tephritid Fruit Flies in Support of Planning and Implementing Area-Wide Integrated Pest Management Programmes (under TC Project RAS5052). 18-22 January 2010, Bangkok, Thailand.

FAO/IAEA/PATTEC Regional Training Course on Standardised Collection and Processing of Entomological and other Relevant Geo-Referenced Data as Needed in SIT-Based AW-IPM Campaigns against Tsetse (under TC Project RAF5060). 1-19 February 2010, Bobo-Dioulasso, Burkina Faso.

FAO International Conference on Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agroindustry to Face the Challenges of Food Insecurity and Climate Change (ABDC-10). 1-4 March 2010, Guadalajara, Mexico.

Fifth Session of the Commission on Phytosanitary Measures, International Plant Protection Convention, FAO. 22-26 March 2010, Rome, Italy.

FAO/IAEA National Coordinators Meeting / Workshop on Area-Wide Management of Fruit Fly Pests, (under TC Project RAS5052). 12-14 April 2010, Muscat, Oman.

31st FAO Regional Conference for Latin America and the Caribbean (LAC). 26-30 April 2010, Panama City, Panama.

Standards Committee Meeting, International Plant Protection Convention, FAO. 26-30 April 2010, Rome, Italy.

FAO/IAEA Training Course on Taxonomía, Ecología y Control de Moscas de Importancia Económica (under TC Project RLA5057). 4-13 June 2010, Panama City, Panama.

FAO/IAEA Regional Training Course on Collection of Baseline Data for the Planning and Implementing of Tsetse Area-Wide Integrated Pest Management in Southern and Eastern Africa (under TC Project RAF5059). 7-24 June 2010, Maputo, Mozambique.

XI Commissioners Meeting of the Panama-United States Commission for the Eradication and Prevention of Screwworm. 8-9 June 2010, Panama City, Panama.

FAO/IAEA National Coordinators Meeting of TC project RAF5059 Supporting the Creation of a Tsetse-Free Zone in Southern Mozambique and North-East South Africa. 28-30 June 2010, Maputo, Mozambique. FAO/IAEA Workshop on Genotyping Analysis of Tsetse Fly Symbionts and Pathogens. 20-24 July 2010, Nairobi, Kenya.

XX Curso Internacional Sobre Moscas de la Fruta. 16-27 August 2010, Metapa de Dominguez, Chiapas, Mexico.

8th International Symposium on Fruit Flies of Economic Importance. 26 September - 1 October 2010, Valencia, Spain. (http://www.fruitflyvalencia2010.org).

FAO/IAEA National Coordinators Meeting / Workshop on Standardised Entomological Monitoring of Old World Screwworm (OWS) Flies (*Chrysomya bezziana*), (under TC Project RAS5054). 28-29 September 2010, Muscat, Oman.

Inception Workshop on Area-Wide Integrated Pest Management of Fruit Flies in South and Southeast Asian Countries. 1-3 September 2010, Bangkok, Thailand.

12th International Workshop of the IOBC Global Working Group on Arthropod Mass-Rearing & Quality Control (AMRQC). 19-22 October 2010, Vienna, Austria. (www.tinyurl.com/amrqcreg)

VI Curso Internacional de Capacitação em Moscas das Frutas de Importância Económica e Quarentenária, 3-11 November 2010, Juazeiro (Bahia) and Petrolina (Pernambuco), Brazil.

FAO/IAEA Workshop on Evaluation of Field Cages for Lepidoptera SIT Behavioural Assessments. 21-22 November 2010, Stellenbosch, South Africa.

Sixth Session of the Commission on Phytosanitary Measures, International Plant Protection Convention, FAO. 14-18 March 2011, Rome, Italy.

Standards Committee Meeting, International Plant Protection Convention, FAO. 2-6 May 2011, Rome, Italy.

Standards Committee Meeting (SC-7), International Plant Protection Convention, FAO. 9-13 May 2011, Rome, Italy.

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 five major topics, namely:

- Fruit Flies
- Mosquitoes
- Moths
- Screwworm Flies
- Tsetse Flies

Country	Project Number	Title	Technical Officer
		National Projects	
Afghanistan	AFG5004	Enhancing Crop Productivity Through Mutation Breeding and Pest Control	Rui Cardoso Pereira
Belize	BZE5002	Establishment of a Pilot Fruit Fly Free Area Using an Integrated Approach that Includes the Area-Wide Sterile Insect Technique	Jesús Reyes
Botswana	BOT5004	Establishment of a Capacity for Integrating the Sterile Insect Technique into the National Tsetse and Trypanosomosis Control Programme	Udo Feldmann
Brazil	BRA5057	Establishment of Mediterranean Fruit Fly, Fruit Fly Parasitoids and Codling Moth Rearing Facility	Rui Cardoso Pereira
Chad	CHD5002	Assessing the Feasibility of Using Sterile Insect Technique Components to Create a Tsetse-Free Zone in the Mandoul Re- gion	Udo Feldmann
Ethiopia	ETH5015	Creating a Tsetse-Free Zone in the Southern Rift Valley	Udo Feldmann
Guatemala	GUA5016	Establishment of Fruit Fly Free or Low Prevalence Areas using the Sterile Insect Technique	Jesús Reyes
Israel	ISR5015	Strengthening the Capacity to Use the Sterile Insect Technique for the Olive Fruit Fly	Jesús Reyes
Jordan	JOR5010	Strengthening the Capacity for the Area-wide Suppression of the Mediterranean Fruit Fly Using the Sterile Insect Technique	Jesús Reyes
Kenya	KEN5022	Integrated Area-wide Tsetse and Trypanosomosis Management in Lambwe Valley	Udo Feldmann
Madagascar	MAG5017	Developing Strategies for Integrated Management of Fruit Flies Based on the Sterile Insect Technique (SIT)	Rui Cardoso Pereira
Mauritius	MAR5016	Feasibility Study for the Suppression of the Melon Fly (<i>Bactrocera cucurbitae</i>) in Selected Areas of Mauritius	Jorge Hendrichs
Morocco	MOR5031	Controlling the Mediterranean Fruit Fly Using the Sterile Insect Technique and Other Conventional Methods	Jesús Reyes
Myanmar	MYA5014	Support for a Feasibility Study on Using the Sterile Insect Tech- nique against Diamond Back Moth	Jesús Reyes

PakistanPAK5043Development of Biological Control for Cotton Pest ManagementJorge HendrichsPanamaPAN5018Maintaining and Operating a Mediterranean Fruit Fly Free Area. Implementing a Fruit Fly Emergency Plan, and SuppressionJesús ReyesSenegalSEN5031Implementing the Pre-Operational Phase to Create a Zone Free of Glossina palpakis gambiensis Using the Sterile Insect TechniqueMarc VreysenSevehellesSEY5003Feasibility of Integrating the Sterile Insect Technique to the On- going Area-Wide Melon Fly Eradication ProgrammeRui Cardoso Percim going Area-Wide Melon Fly Eradication OrgammeSouth AfricaSAF5011Refining an Integrated Application of SIT Against Some Key Lepidopteran Pests of Southern African Agricultural CropsJorge HendrichsSudanSUD5032Investigating the Use of Inherited Sterile Insect Technique for Con- rolling Mosquitoes in Northern SudanJeremie GitlesTunisiaTUN5026Assessing the Use of Inherited Sterility as a Genetic Control Method against the Carob MothMarc VreysenRafficianRAF5039Supporting the Creation of a Testes-Free Zone in Southern Mozambique and North-East South AfricaMarc VreysenRafricanRAF5050Supporting the Use of the Sterile Insect Technique for Area Wide Testes and Trypanosomosis Management (Phase II)Udo FeldmannAfricaRAS5051Developing Integrated Control of the Olive Fruit Fly Mozambique and North-East South AfricaMarc VreysenRafrianRAS5051Developing Integrated Control of the Olive Fruit Fly Pest Man agement ProgrammesJestis ReyesRegional Asia<				
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Highlights of Technical Cooperation Projects

Implementing the Pre-Operational Phase to Create a Zone Free of *Glossina palpalis gambiensis* Using the Sterile Insect Technique (SIT) (SEN5031)

A delegation of four people from the Mubarqui company visited the project SEN5031 in April to discuss the development of a chill fly aerial release system for tsetse. The delegation consisted of Ruben Leal-Mubarqui, owner of the company, Roberto Angulo, Rene Cano Perez and Jose Luis Zavala, fruit fly release expert from the Mexican National Fruit Fly Campaign. This team is developing a chill fly aerial release system suitable for tsetse together with navigation equipment, recording and management software as a donation to the Senegal project. The system is based on the system used for fruit fly releases in Central America, but has been extensively modified to fit the much lower release rates and much higher cost of tsetse flies compared to fruit flies.



Gyrocopter to be used in aerial releases of Glossina palpalis gambiensis in Senegal.

Together with the project counterparts, Baba Sall, Momar Seck and Jeremy Bouyer, the company Domain de Kalahari was visited, which is proposed to do the aerial releases. This company has a number of two seat (tandem) gyrocopters that they use for sightseeing trips for tourists. It is proposed that these gyrocopters would be suitable for tsetse fly release. The Mubarqui team examined the gyrocopters with a view to fitting the chill release machine into the rear passenger compartment with the necessary associated power supplies and navigation equipment. They informed us that the new system would be ready in five months' time (September 2011). The Mubarqui team also demonstrated the MacX web-based management system for recording and monitoring all aspects of the fruit fly release programmes in Central America. The system records rearing, release and monitoring data and presents these in various formats, including via Google

Earth allowing an immediate visual impression of the area covered and the accuracy of the ongoing releases.

Any errors in track or altitude are immediately visible and a 'virtual' flight can be followed to see the release path. Ruben Leal has offered to modify the MacX system, which is based on MySQL, and make it available for the tsetse project's use. The current project database is in the process of being converted to MySQL and Rene demonstrated how easily and quickly the data could be loaded into the MacX system. It is recommended that the MacX system be adopted for the project, with suitable modifications appropriate to tsetse biology.

An initial contract for test releases will cover about 60 hours total flying time in the Kayar area, including a mobilization component to cover obtaining permits, preparing the gyrocopter for the releases, identifying and preparing emergency landing areas in the release zone, etc. As part of this test shipments of pupae will be sent from Seibersdorf or Bratislava for comparison with the quality of the pupae shipped from 'Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide' (CIRDES) in Burkina Faso. The first shipment will be made to arrive in Dakar in May for release in Kayar simultaneously with the release of the CIRDES flies so that a direct comparison can be made. Depending on the outcome of this shipment a second shipment will be sent four weeks later and possibly further shipments.

Creating a Tsetse-Free Zone in the Southern Rift Valley (ETH5015)

FAO/IAEA continued to provide technical assistance to the Southern Tsetse Eradication Project (STEP). This large and complex project aims at controlling and eventually eliminating the tsetse and trypanosomosis problem in the Ethiopian Southern Rift Valley by developing and applying an area-wide integrated pest management (AW-IPM) approach, involving a sterile insect technique (SIT) component. The project has passed the phases of baseline data collection and feasibility assessment and is currently in the pre-operational and capacity building phase. In order for STEP to enter and receive support for the operational phase, some ten identified shortcomings, relevant to project management and a few of a technical nature, need to be addressed before completing the preoperational phase in mid-2012.

By May 2011 'good' and 'some progress' could already be reported on three and six of these shortcomings, respectively. Worth mentioning in this context are:

- a) a revised project management set-up and the appointment of a new, effectively working project management team;
- b) a substantial increase of the number of mass-reared tsetse colony females, needed to supply sterile male flies for aerial release in SIT operations: the *Glossina*

fuscipes fuscipes colony has meanwhile exceeded 600 000 colony female flies (see Figure below);

c) progress in the initiation of intensive and area-wide pre-SIT tsetse population suppression in the entire 10 500 km² STEP 'block-1' field area, using insecticides on targets and on livestock, as well as in the near future, involving the sequential aerosol technique (SAT).

Preparations are under way to initiate pilot operational aerial releases of sterile *G. f. fuscipes* male flies in late 2011 in the Deme valley.



Number of mass-reared tsetse colony of Glossina fuscipes fuscipes (females) at Kaliti mass-rearing facility.

Assessing the Feasibility of Establishing a Tsetse Free Zone in Lake Victoria Basin (UGA5031)

Collaborators in Uganda have made progress with standardised collection and processing of field data relevant to the tsetse and trypanosomosis problem. Tsetse suppression activities are under way in large parts of the *Glossina fuscipes fuscipes* belt in the country. A Government request to the IAEA for technical assistance against this key vector of animal and human African trypanosomosis in the country beyond 2011 was a basis for a meeting, held in IAEA Vienna in late-May 2011 between tsetse control specialists from Uganda and Ethiopia. In the past years Uganda has repeatedly faced difficulties with creating a mass-reared colony of *G. f. fuscipes*. This prevented the development of a tsetse SIT component as part of AW-IPM measures in the country.

As the STEP mass-rearing facility in Ethiopia meanwhile appears to be in a position to produce substantially more sterile male flies of this species than the SIT operations in the STEP project area require, Ethiopia and Uganda entered negotiations and elaborated detailed plans for the procurement of sterile males for an SIT feasibility demonstration in a pilot area in Lake Victoria in Uganda, which is proposed to be implemented in the TC biennium 2012-2013.



Raising awareness among rural communities on tsetse control activities (Photo by: F. Luyimbazi).

Investigating the Feasibility of Using the Sterile Insect Technique for Controlling Mosquitoes in Northern Sudan (SUD5032)

A meeting was convened from 18 to 21 April 2011 in Vienna (Austria) with the different stakeholders of the mosquito project in Northern State, Sudan including the Islamic Development Bank (IDB); the Government of Sudan represented by Tropical Medicine Research Institute (TMRI), the Sudan Permanent Mission in Vienna, and the International Atomic Energy Agency (IAEA) to discuss the current status, future requirements and potential strategies. This project is supported by the IAEA through technical cooperation project SUD/5/032 – 'Investigating the use of sterile insect technique (SIT) for controlling mosquitoes in the Northern Sudan'.

More specifically, the meeting discussed the required adjustments of the overall project workplan, assessed present and future financial resources being contributed by the IDB, IAEA and the Government of Sudan and agreed on the respective responsibilities of each of the stakeholders of this collaborative partnership.

During the meeting, strategic and logistical aspects of the project were discussed such as the selection of an appropriate site for the construction of the mass-rearing facility, the scope, size and site of the pilot trial, the sustainable production of approximately 500,000 sterile males of good quality per week during implementation of the pilot trial, as well as some other crucial issues.

The meeting recommended that the project implementation adheres to the phased conditional approach, which implies a mandatory completion of all activities in the respective phase before proceeding to the next phase. The coordination meeting also highlighted that current resources are inadequate and several million US dollars would be needed to complete and operate the massrearing facility and to implement the field pilot trial.

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

Project Number	Ongoing CRPs	Scientific Secretary
G3.40.01	Development of Standardised Mass-Rearing Systems for Male Mosquitoes (2005-2011)	Jeremie Gilles
D4.20.12	Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens (2007-2012)	Adly Abd Alla
G3.40.02	Biology of Male Mosquitoes in Relation to Genetic Control Programmes (2008-2013)	Jeremie Gilles
D4.20.13	Applying Population Genetics and GIS for Managing Livestock Insect Pests (2008-2013)	Udo Feldmann
D4.10.22	Increasing the Efficiency of Lepidoptera SIT Through Enhanced Quality Control (2009-2014)	Marc Vreysen
D6.20.08	Development of Generic Irradiation Doses for Quarantine Treatments (2009-2014, managed with Food and Environmental Protection Subprogramme)	Andrew Parker (co-secretary)
D4.20.14	Development and Evaluation of Improved Strains of Insect Pests for SIT (2009-2014)	Gerald Franz
D4.10.23	Resolution of Cryptic Species Complexes of Tephritid Pests to Over- come 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)	Rui Cardoso Pereira

The Final RCM of the CRP on Development of Standardized Mass-Rearing Systems for Male Anopheles arabiensis Mosquitoes. 7-11 March 2011, St. Clotilde, La Réunion, France

The final Research Coordination Meeting of a Coordinated Research Project (CRP) on 'Development of Standardised Mass-Rearing Systems for Male Anopheles arabiensis Mosquitoes' was held in La Reunion, France in March 2011. During the five years of the CRP, significant progress was made with the development and validation of new mass-rearing tools for mosquitoes. A trayrack system was developed for Anopheles arabiensis and tested for Aedes albopictus. A novel device, called the larval pupal separator, was developed to separate larvae and pupae of Anopheles arabiensis. The new equipment was validated and was capable of separating a mixture of one million larvae-pupae in one hour. An affordable and performing larval diet is now available and is contributing to the establishment of new colonies in the laboratory. New mass-rearing procedures have likewise been

developed. Sterilization procedures using gamma ray and X ray irradiators were also developed for both *Anopheles arabiensis* and *Aedes albopictus*. All these findings and progress will be published in an IAEA-TECDOC.



Participants of the final RCM of CRP on Development of Standardized Mass-Rearing Systems for Male Anopheles arabiensis Mosquitoes (St. Clotilde, La Réunion, France).

The Second RCM of the CRP on Development of Generic Irradiation Doses for Quarantine Treatments. 11-15 April 2011, College Station, TX, USA

The second RCM of the CRP on the 'Development of Generic Irradiation Doses for Quarantine Treatments' was hosted by the E-Beam facility of Texas AgriLife Research in College Station, Texas. The meeting was opened by Craig Nessler, Director of Texas AgriLife Research, who welcomed the participants. The meeting was attended by six contract holders, five agreement holders and five observers. Five contract holders were unable to attend.



Participants of the second RCM of CRP on Development of Generic Irradiation Doses for Quarantine Treatments (College Station, TX, USA).

The meeting reviewed the progress towards the objective of establishing generic doses for several arthropod groups. Good progress has been made in several groups and it is expected that confirmatory tests will be completed by the next RCM for mealybugs, *Liriomyza* leaf miners and some Lepidoptera. Tests may also be completed for two species of mites. With the exception of the mites, the results so far indicate that generic doses significantly below the currently proposed 400 Gy for all insects except pupae and adults of Lepidoptera will be appropriate for each of these groups. The dose for the mites is still not clear as there seems to be considerable variation between species.

The results of the dose comparison scheme were presented. For all but one of the participants of the CRP who submitted dosimeters for reading, the correspondence between the target dose and the dose measured by the test dosimeters was very good, falling within the $\pm 5\%$ confidence interval of the dosimetry system.

Abdeljelil Bakri presented to the meeting a prototype of a new online resource developed by the Insect Pest Control Section (IPCS) for communication among participants of the CRP. This was enthusiastically received with many suggestions for improvements and expansions. This on-line resource, and others that are under development, will be soon placed under our IPCS website.

The Second RCM of the CRP on Development and Evaluation of Improved Strains of Insect Pests for SIT. 9-13 May 2011, Nanjing, China

The second Research Coordination Meeting of the Coordinated Research Project on 'Development and Evaluation of Improved Strains of Insect Pests for SIT' was hosted by Nanjing Agricultural University in Nanjing, China.

This CRP was initiated as a result of a recommendation from a Consultants Group Meeting held in Antigua, Guatemala, in August of 2008. The consultants recommended that the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture transfer existing technologies for genetic sexing strategies to key insect pests; develop marker strains for use in the field that will enable the tracking of released insects especially with regard to their ability to mate with field females; develop stable transgenic lines that will improve the effectiveness of the SIT, and encourage the extension of this technology into lepidopteran pest species. The major outcome of the use of these improved strains will be a more cost-effective and efficient implementation of the SIT in area-wide programmes against major insect pests of agriculture and human health.

Twenty-four scientists, including eight observers, from fifteen countries attended this second RCM. The participants reported on the progress made since the last RCM in 2009. Specifically the following four areas were considered: 1.) insect transformation, 2.) sex determination, 3.) alternative sexing systems and 4.) evaluation of existing strains.



Participants of the second RCM of CRP on Development and Evaluation of Improved Strains of Insect Pests for SIT (Nanjing, China).

Significant progress has been made in the application of genetics and molecular biology to control pest insects. Technologies have also been transferred to additional species. Strains are now available for larger scale evaluation and, if evaluated positively, for field application.

Developments at the Insect Pest Control Laboratory (IPCL), Seibersdorf

FRUIT FLIES

Fruit Fly Rearing and Quality Control Group Activities

Mating Compatibility Studies with Members of the *Bactrocera dorsalis* Cryptic Species Complex

Mating compatibility studies of various members of the cryptic species complex of *Bactrocera dorsalis* have been pursued. These studies have the objective to resolve the disputed taxonomy status in order to facilitate international trade of fresh fruits and vegetables and to assess whether the sterile insect technique (SIT) can be applied and enhanced for this species complex.

Mark Schutze, a visiting scientist from Queensland University of Technology, Brisbane, Australia, worked on various identification tools to distinguish species within the *B. dorsalis* complex. He conducted cross-mating studies in field cages between *B. dorsalis* (Saraburi Thailand), *B. carambolae* (Suriname), *B. philippinensis* (Philippines) and *B. papayae* (Malaysia).

Relatively high levels of mating isolation were obtained with those mating combinations that included *B. carambolae*. All other combinations among the other three entities, i.e. *B. dorsalis*, *B. papayae*, and *B. philippinensis* exhibited random mating.

Effect of Methyl Eugenol on Mating Compatibility between *Bactrocera dorsalis* and *B. carambolae*

Methyl eugenol (ME) is a naturally occurring phenylpropanoid found as a plant secondary metabolite and a component of essential oils in many plant species. Consumption of ME by male *B. dorsalis* and *B. carambolae*, a known precursor to pheromone production, has been reported to enhance their mating success. Therefore, the effect of ME feeding on male mating success in cross mating studies under field cage conditions was studied.

The objective of these studies was to assess the effect of ME feeding and non-feeding and whether ME feeding could minimize or remove the mating isolation between *B. dorsalis* and *B. carambolae. Bactrocera dorsalis* sourced from Saraburi, Thailand and *B. carambolae* sourced from Suriname were used for the cross-mating studies. The males (n=100) were allowed to feed on ME (0.5 mL of ME laced on 20 by 5cm filter paper strip) for one hour and their mating success tested two days later.

The results so far showed that ME-feeding increased the mating rate, but did not reduce their mating isolation. Further ME and cross-mating studies among *Bactrocera* spp. are on-going in collaboration with Todd Shelly from USDA in Hawaii.





Bactrocera dorsalis (top) and B. carambolae (bottom) males feeding on methyl eugenol.

Olive Fruit Fly (Bactrocera oleae) Rearing

Research on improving the mass-rearing of olive fruit fly for SIT programmes continued. One of the main challenges was the development of efficient egg collection methods that were suitable for a mass-rearing set-up. The development of these egg collection methods has gone through a series of stages using wax domes (A), wax cones (B) and wax coated cloth (C) enabling the rearing of olive fly on a small scale in small bench type cages or medium sized cages. The next step was to upscale the rearing and to use bigger cages suitable for mass-rearing purposes. Efforts in 2010 resulted in cultures of olive flies maintained in large cages used for Mediterranean fruit fly mass-rearing that had wax coated cloth panels (D) for oviposition. During eight generations, the olive fly cultures produced high numbers of eggs, but as the wax-coated cloth had certain limitations, i.e. difficult to prepare and fragile, this set-up was not ideal for massrearing purposes. The final step was to progress towards rearing in mass-rearing cages that have normal cloth mesh (E). To date, the maximum amount of eggs collected from such a cage was 91 mL or 3.1 million olive fly eggs over a period of 3 weeks. The trend in production efficiency continues to go upward indicating that olive fly mass-rearing is becoming a reality.



Evolving process on collecting eggs in the mass-rearing of olive fruit fly (A) wax domes (B) wax cones (C) wax coated cloth (D) wax coated cloth panels and (E) normal netting.

Fruit Fly Genetic Sexing Group Activities

Evaluation of GSS Carrying a Balancer and the Marker Sr^2

Genetic sexing strains (GSS) ideally carry an easily identifiable marker to allow discrimination of the released flies from the wild flies in the target population. For the Mediterranean fruit fly two different marking strategies exist, excluding those based on transgenesis: the use of a specific mitochondrial haplotype or the use of a phenotypic mutation. For the latter the marker Sergeant 2 (Sr^2) was developed (see NL 69). Sr^2 is located at 60D on the trichogen map, i.e. in the vicinity of white pupae (*wp*, 59B) and the temperature sensitive lethal (*tsl*, 60B-61B) that are used as selectable markers in the current GSS like VIENNA 8. The mutation is lethal in a homozygous condition. Three different GSS, utilizing Sr^2 as marker, were constructed (Table below) and compared to the original VIENNA 8.

VIENNA 8- Sr^2 was created by screening large numbers of flies for a recombination event between the Yautosome translocation T(Y;5)101 and a free autosome carrying Sr^2 . As a result a Y-autosome translocation is available that allows the males of the GSS to be distinguished from the wild population. The strain GS-9 was constructed by irradiating a balancer chromosome for chromosome 5 (labelled with Sr^2) produced by A. Zacharopoulou (University of Patras). This balancer chromosome was generated by three consecutive rounds of irradiation resulting in three overlapping inversions that cover virtually the entire length of chromosome 5 (labelled 223, 86 and 29 in the Figure below).



Schematic representation of the salivary gland polytene chromosome 5. The upper panel shows the stepwise construction of the balancer chromosome and the inverted areas are indicated by the blue line in comparison to the uninverted chromosome (SG). The lower panel shows the inversion in D53. Some important markers are also shown: w (white), y (yellow body), wp (white pupae) and Sr^2 (Sergeant 2).

Strain	Translocated chromosome	Phenotype males	Phenotype females	Translocation breakpoints	Free autosome
VIENNA 8	Wild type	$wp^+ tsl^+$	wp tsl	52B	D53 (wp tsl Sr^{2+})
VIENNA 8-Sr ²	Wild type	$wp^+ tsl^+ Sr^2$	wp tsl Sr^2	52B	D53 (wp tsl Sr^{2+})
GS-9	Balancer	$wp^+ tsl^+ Sr^2$	wp tsl Sr^{2+}	49C	wp tsl Sr^{2+}
GS-9/D53	Balancer	$wp^+ tsl^+ Sr^2$	wp tsl Sr ²⁺	49C	D53 (wp tsl Sr^{2+})

Summary of the structures and phenotype of the strains used

Inversions are used to avoid the consequences of recombination in the heterozygous males in a GSS which leads to breakdown of the sexing system. Recombination events occurring within the inversion lead to genetically unbalanced offspring which are not viable. To link the Y chromosome to the balancer males, heterozygous for the balancer, were irradiated with 40 Gy followed by mass mating with *w wp* females.

With F1 males showing a Sr^2 + phenotype 100 single pair crosses with *w* wp females were set up. The F2 was screened for families where females are *w* wp and the males are $w + wp + Sr^2$. One family was recovered with a translocation breakpoint at 49C (trichogen map) which is equivalent to 67C on the salivary gland map. This strain was either combined with the regular wp tsl chromosome or with the inversion D53 (Table previous page).

Strains were maintained for 48 to 80 generations without removing any recombinants. Each generation 34 mL of pupae were used to set up the next generation while a parallel sample of 40 mL was screened. Table below shows a summary of the results.

Results of the long term reading of four GSS. The recovery of adults per 40 mL of pupae is shown only for fully emerged and non-crippled individuals. Recombination frequencies are shown for either the interval between the translocation breakpoint (B) and wp or Sr²

Strain	Generations reared	Males/ 40 mL	Females/ 40 mL	Recombina- tion frequency (B-wp)	Recombination frequency (B-Sr ²)
VIENNA 8	80	1343	974	0.0141	N/A
VIENNA 8-Sr ²	52	1286	1009	0.0085	0.0124
GS-9	48	1083	951	0.0038	0.0085
GS-9/D53	48	1107	1021	0.0037	0.0155

At this level of rearing the VIENNA 8- Sr^2 strain shows a moderate reduction in productivity and a slight improvement in stability. Both GS-9 strains show a more pronounced reduction in productivity which is to be expected as the this translocation does not have the very specific features of the one used in VIENNA 8 (= T(Y;5)101). The recombination frequency in the interval between the translocation breakpoint and *wp* is very low. In the case of GS-9 in combination with the regular *wp tsl* chromosome also the recombination between breakpoint and Sr^2 is reduced. However, if D53 is combined with the balancer translocation the recombination is relatively high, potentially due to the fact that two inversions are involved so that recombination reducing effect is cancelled out.

TSETSE FLIES

Salivary Gland Hypertrophy Virus

As reported in the last newsletter (NL 76, January 2011), attempts are continuing to develop a strategy to manage the Salivary Gland Hypertrophy Virus (SGHV) that can hamper the development of *Glossina pallidipes* colonies due to reduced productivity. The virus management strategy is based on four approaches, (1) blocking virus replication using commercial antiviral drugs, (2) inhibiting virus infection by silencing virus specific genes using RNAi technology, (3) neutralizing the virus infection us-

ing virus specific antibodies, (4) modifying the feeding system to clean blood feeding and (5) impeding the SGHV infection using a peptide similar to SGHV ORF005 that is hypothesized to bind to the gut epithelium.



Henry Kariithi from Kenya assessing the possibility of neutralising the SGHV infection using specific antibodies

Work is continuing to assess the impact of the antiviral drug Valacyclovir on the viral infection in *G. pallidipes* and to assess its long-term toxicity. So far, after 30 months of using Valacyclovir at 300 μ g/mL, the results indicate a significant reduction in the prevalence of salivary gland hypertrophy (SGH) symptoms (close to complete elimination of the syndrome) in the treated flies while the non-treated flies continue to show a higher SGH prevalence. Moreover the treated flies continue to maintain acceptable levels of productivity and mortality which permit an increase in the colony size. This result strongly supports our recommendation to use Valacyclovir to reduce the SGHV in *G. pallidipes* colonies.

Based on the results obtained with Valacyclovir, another 15 antiviral drugs currently used against herpesviruses were selected to test their effect on virus replication. So far, the toxicity of three of these drugs to tsetse flies has been tested and the results indicate that two of the tested drugs (Fluoruracil and Trifluridine) are very toxic to the flies and caused very high and rapid mortality. In contrast the third drug (Tenofovir) did not show severe negative effects on the flies (with an acceptable level of productivity and very low mortality). In addition, dissection of the treated flies showed that Tenofovir caused significant reduction in SGH prevalence in the treated flies and their progeny. Further studies on Tenofovir will be required to assess the impact of long term administration of this drug to the flies.

Work is continuing to optimize the use of RNAi technology to silence some virus specific genes to inhibit virus infection. An experiment has been started to assess the effect of suppressing the expression of p74 by RNAi on virus infectivity. No significant results have so far been obtained on fly productivity and mortality and the prevalence of the SGH between the RNAi treated and untreated flies. Moreover some technical problems were observed in relation to the plasmid's stability in the transformed bacteria. Work is continuing to resolve these problems and to test more virus genes using this technology. More than 30 genes have been cloned and sequenced and their impact on viral replication is waiting to be tested.

For neutralization of the virus infection using virus specific antibodies, five antibodies were produced. Experimental work to assess the efficacy of the antibodies to neutralize and block virus infection has been started. The preliminary results showed that injecting virus neutralized by antibodies against ORF 10 into tsetse flies significantly reduced virus infectivity. Moreover, adding p74 antibodies to the blood contaminated by feeding symptomatic infected flies significantly reduced the virus load compared to flies fed on contaminated blood without antibodies. Optimizing and assessing the antibody neutralization effect in more details on the virus is continuing.



SGHV virus attach specifically to tsetse midgut. Midgut tissue observed under normal light (A) and under UV light (B).

After demonstrating the role of horizontal transmission through the membrane feeding system used in laboratory colonies, it was recommended to maintain tsetse flies on a clean blood feeding system to provide additional evidence that feeding flies clean blood is capable of reducing the virus load in the colony and to determine the time needed with clean feeding to achieve an acceptable reduction in the virus load in the treated colony. A clean feeding colony was established by putting fly cages on sterile membranes and providing blood to the flies that had not been used to feed any other flies. After feeding, the remainder of the blood was used to feed the remainder of the colony. The clean feeding colony was maintained separately from the other colony and fly samples were taken regularly to assess the virus load and the prevalence of SGH.

The qPCR results indicated a significant decrease in virus load in the clean feeding colony in comparison with the standard feeding colony. Fly dissections indicate that after nine months the clean feeding colony became virtually SGH free while 10% of the flies in the normal feeding colony still showed SGH. The virus prevalence by PCR on the leg of teneral flies indicated that the clean feeding colony continued to have low virus prevalence. In addition, the flies fed permanently on the contaminated blood previously used to feed the clean feeding colony showed an increasing rate of SGH, which in some cases reached 50%. This result, in addition to the reduction of the SGH in the clean feeding colony, provides strong evidence that horizontal transmission of the virus through the blood feeding is the most important route for virus contamination in laboratory colonies of tsetse flies.

Recently, a new strategy was introduced to control SGHV by impeding the SGHV infection using a peptide similar to the SGHV ORF005 that is hypothesized to bind to the gut epithelium. This work was started in collaboration with Bryony C. Bonning from Iowa State University, Ames, USA who demonstrated the efficiency of this strategy in reducing the infection of a baculoviruses in lepidopteran larvae. This strategy is based on occupying the virus receptor in the epithelium of the midgut (where the virus should attach to be able to start the infection process) using an oligo-peptide similar to the polypeptide found in the virus genes.

One polypeptide sequence similar to the amino acid sequence used by B. Bonning was found on SGHV ORF005. An oligo-peptide was synthesized from this sequence and its impact on virus infection was tested. The preliminary results indicate a significant reduction in the virus load in the flies fed on the oligo-peptides with the virus compared to the flies fed on the virus alone.

Further studies to optimize and assess the impact of longterm use of this approach are under way. To ensure a sustainable and economically acceptable way to produce the oligo-peptides, we started to screen a phage display library in collaboration with B. Bonning, which expresses a wide range of oligo-peptides to select one phage colony which expresses a peptide similar to the viral binding sequence. For this, Sijun Liu from Iowa State University visited the IPCL to start screening the phage library and to train the laboratory staff to continue this work.



Visiting scientist Dr Sijun Liu from Iowa State University, USA collaborates with IPCL staff on the screening of phage display library in tsetse flies.

Field Cage Tests

Field cages are used to provide a more natural environment for observing insect behaviour, and in particular mating behaviour and competitiveness. Two sets of field cage experiments were conducted recently in the laboratory.

The first set was to investigate any potential barrier to free mating between the wild population of *G. palpalis gambiensis* from Senegal, which has recently been colonized in Seibersdorf, and the long-standing colony of this species from Burkina Faso maintained at CIRDES, Bobo Dioulasso. The test showed that there is no barrier between these colonies, despite them originating from widely separated locations.

In the second set of experiments, the impact of SGHV infection on male *G. pallidipes* performance in terms of mating success and the mating behaviour of wild females mated with the infected males was investigated. The results indicate that males with SGH are less successful in mating when competed with males without SGH. Also, a high percentage of females mated with males with SGH have empty spermathecae. Although most of the females mated with males did not show any increased tendency to remate with males with normal salivary glands.

Dose Comparison Scheme

The IPCL initiated a dose comparison scheme in late 2009. The purpose of this service is to provide a level of confidence in radiation dose measurement for SIT and quarantine irradiation projects where the researchers involved have limited or no access to dosimetry services and must perform their own dosimetry. A test and a control dosimeter set were provided on request to be irradiated during routine operations and returned to the IPCL for reading. The dosimeters are not transfer standard dosimeters and are not suitable for calibration.

Forty dosimeters were sent out of which 23 were returned for reading. The results presented in the graph show that most of the measured doses were within an acceptable range of the target dose. A few measurements show significant deviation from the target and further follow-up is required to determine the reason for this discrepancy.



Measured dose and estimated dose from 23 dosimeter readings in 2009 and 2010.

MOSQUITOES

Effects of Sterilization on the Sexual Maturation Period of Male *Aedes albopictus*

The effect of irradiation on the time required for sexual maturation in male *Ae. albopictus* was studied by observing the rotation of the genitalia during the first 30 h after emergence for both wild males and males irradiated as pupae with 35 Gy (95% sterile). The genitalia of male mosquitoes are rotate 180° after emergence and this natural process can be divided into five stages.

The statistical comparisons showed that in un-irradiated males, rotation started slightly later than in those irradiated. However, the contrary effect was observed at the end of the process as irradiated males were slightly slower to complete rotation. Overall, the effect of irradiation on the rotation rate of the genitalia was minimal and would not be disadvantageous in the context of SIT.

The insemination status of mature virgin females left together with emerged males aged 10 to 15 h old showed that no insemination occurred even though mating attempts were observed. Rotation of genitalia is not the exclusive prerequisite governing sexual maturity; hence 15 h old males with terminalia fully rotated might not yet be able to transfer sperm. Further work is ongoing in relation to mating ability of wild and laboratory-reared and irradiated male *Ae. albopictus*.



Evolution of Aedes albopictus male genitalia rotation with age. Stage 0 (no rotation) to stage 4 (rotation completed) are shown. Percentage of males in the different stages for un-irradiated males (top) and males irradiated at 35 Gy (bottom). Due to a lack of irradiated mosquitoes, no samples were analysed after all rotated completely (20 hours), except at the end of the experiment.

Radiation Induced Sterility on Male Aedes albopictus from Reunion Island

The relationship between irradiation dose and sterility of an *Ae. albopictus* strain from La Réunion was studied using a gamma-cell irradiator with a Cesium-137 source. Full sterility was obtained with a dose of 40 Gy, and the radiation induced sterility was consistent with the one obtained at the IPCL with Cobalt-60 and X ray irradiation of the Bologna strain of *Ae. albopictus*.

The persistence of sterility over successive matings was assessed. Two mating periods of five days, during which one male was confined with 10 females, were interrupted by a resting period of 5 days to allow the male to produce new sperm cells. For each male, one to 6 females were able to lay eggs after blood feeding. The mean sterility of these females (N=22) was $97 \pm 0.6\%$ (mean \pm se) during the first mating period. However after the resting period, the sterility was complete for all females (N=17) inseminated by males during the second mating period. No recovery of fertility occurred after matings and after a resting period. On the contrary, the sterility of males was even higher during successive matings. The irradiation process as carried out here ensured permanent sterilization of the males.

Competitiveness of Sterile vs. Wild Male Aedes albopictus under Semi-Field Conditions on Reunion Island

Competitiveness tests are fundamental in assessing the quality of the sterile males that are to be released for the SIT. In order to obtain a reliable indicator of competi-

tiveness, these tests have to be performed in an environment that mimics as closely as possible natural conditions. In this context, experiments were conducted in semi-field conditions on Reunion Island (see following figure). Males irradiated with 35 Gy (dose conferring 95% sterility) were in competition against wild males for mating with wild females for 7 days.



Semi-field settings for mosquito competitiveness studies on Reunion Island.

When males were released in the cage on the day following emergence, with an equal ratio of wild and sterile males, the competitiveness index (CI), which can range from 0 to 1, was low. However, when males were kept 5 days in the laboratory before release in the field cages, CI values ranged between 0.59 and 0.82. This indicates an almost equal participation of both groups of males in the insemination of females. Either the age of males or the time spent in the insectary before release may have enhanced the competitiveness of irradiated males. Increasing the ratio of sterile to wild males (released when one day old) reduced the mean fertility of the population.

These results confirm that release of sterile *Ae. albopictus* should be done with a higher ratio of sterile males as compared to the density of wild males in the field. Moreover, allowing a period of rest to the sterile males might benefit the efficiency of the releases.



Competition of two Aedes albopictus males for mating with a female (photo by: Ludovic Laï-Yu).

In order to understand the differences between releases of one- or five-day old males, tests were carried out to study the mating success of sterile males aged 1 to 5 days old or 5 to 10 days old. The mean number of inseminated females was not significantly different during the two periods. However these tests were done under laboratory conditions and it is likely that the differences in male performance in relation to their age only became apparent under semi-field conditions.

Mating Success of Sterile Male Aedes albopictus

The insemination capacity of sterile male *Ae. albopictus* (irradiated at 40 Gy) was compared to that of untreated males. Four-day old sterile males inseminated as many females as untreated males of the same age during a 48 h period. However, when males were given new 10 females each day for 15 days, the insemination success of sterile males was lower as compared to untreated ones but this difference was significant only after the 9th day (see the following figure).



Mating success of un-irradiated and irradiated Aedes albopictus males. Mean number of females inseminated after different periods by one untreated or one sterile male. (*) indicates a statistically significant difference P < 0.05.

The mean number of females inseminated daily by a sterile male was always lower as compared to an untreated male. *Ae. albopictus* females have 3 spermathecae where the transferred sperm is stored; when mated by sterile males, females generally had only one spermathecae filled, whereas females mated by untreated males had one to three spermathecae filled (see following figure).



Mean number of spermathecae filled by one male Aedes albopictus. Each male, either untreated or treated, was together with 10 new virgin females each day.

Sperm Production of *Anopheles arabiensis* with Respect to Genetic Manipulation, Dieldrin Application and Irradiation

Before their release, several handling procedures are imposed on male Anopheles arabiensis. From the original strain, a genetic sexing strain (GSS) based on a dieldrin resistant mutation was created by translocation. Using this GSS, dieldrin treatment (low dose/low volume) of the eggs allows a complete elimination of female mosquitoes mostly at the L1 stage. This step is crucial as we cannot afford to release female mosquitoes which are the vectors of malaria (males do not feed on blood and are hence harmless for humans). Furthermore, the absence of female larvae and pupae reduces costs, space and labour requirements during mass-rearing. After development, the male pupae are sterilized by irradiation. The impact of genetic manipulation, dieldrin treatment, and gammairradiation (at 70 Gy) on sperm number and sperm production was studied separately and together.

Five groups of males were tested and examined for sperm counts: Dongola males, GSS males, GSS males irradiated at 70 Gy, GSS males treated with dieldrin and GSS males treated with dieldrin and irradiated at 70 Gy. The males were examined for sperm production when two days (for initial sperm count) and six days old (sexually mature males) (see Table).

Sperm count in Anopheles arabiensis males at the age of 2 and 6 days after exposure to different treatments.

Treatment	Male age			
Treatment	2 days	6 days		
Dongola males	3232.3 ± 256.5	4395.8 ± 304.5		
GSS males	3123.4 ± 246.8	4758.9 ± 242.8		
70 Gy irradiated GSS males	1933.2 ± 200.8	1069.9 ± 222.8		
Dieldrin treated GSS males	1877.0 ± 173.3	3608.3 ± 333.8		
Dieldrin treated and 70 Gy irradiated GSS males	1886.1 ± 209.1	-		

Dongola and GSS strains showed a similar pattern, i.e. same initial sperm count and same production of sperm throughout adult life, while the irradiation and dieldrin treatments appear to have an impact on the initial sperm count, which was lower than in untreated Dongola and GSS males. Moreover, for the irradiated males, there is no sperm production and furthermore there is a decrease of the number of sperm with age. In the dieldrin treated males, despite a lower number of the initial sperm count, the production of sperm did still occur.

Finally, for the males that are to be released in a SIT programme, dieldrin treated and 70 Gy irradiated, the initial sperm numbers were comparable to those of males after irradiation or dieldrin treatment. In other words, there seems to be no cumulative effect of the two treatments on initial sperm numbers and sperm production. More replicates will be needed to allow proper statistical analysis.

Effect of Sugar Supply on the Survival of Irradiated Male Anopheles arabiensis and Aedes albopictus

For male mosquitoes, sugar is essential at the beginning of adult life to increase longevity and to achieve an optimal mating period. In the absence of sugar, survival of males is dependent solely on the accumulation of larval reserves.

To increase the efficiency of sterilized males released in the field, offering a sugar supply at the beginning of the adult life before release could be a way to increase survival and the probability of mating.



Adult survival of a) Anopheles arabiensis and b) Aedes albopictus males under different feeding schedules. For the treatments no water and water, day 0 corresponds to the day the males emerge. For sugar treatment, day 0 corresponds to the day of sugar removal.

Male *An. arabiensis* and *Ae. albopictus* pupae were irradiated at 70 Gy and 40 Gy respectively. For each species, newly emerged males were divided into 15 groups of 50 individuals. Each group was then placed into a standard cage (30 by 30 by 30 cm). Three groups received no water and no sugar. Three groups were supplied with only water. The remaining groups had access to a 10% sucrose plus 0.2% methylparaben solution for either 24 h, 48 h or 72 h (3 groups each). Survival was recorded daily.

Giving sugar to laboratory males before release did not increase survival and suggests that in the field, the capacity to inseminate wild females will depend largely on the presence of a sugar source in the environment. The importance of water for the survival of *Ae. albopictus* still needs to be further studied.

A Comparison of Three Artificial Diets for the Rearing of *Aedes albopictus*

Recent research on *Aedes albopictus* focused on optimizing current mass-rearing practices, specifically in the area of larval diets and nutrition in support of the ongoing sterile male release programme of the Centro Agricoltura Ambiente (CAA) in Bologna, Italy. Larval diet is of critical importance as it has major influence on adult mosquitoes.

Three mosquito-specific diets for the rearing of *Ae. al-bopictus* were tested. The first diet called CAA diet, was developed by the CAA and consists of ground cat food (Friskies[®]), brewer's yeast and fish food (Tetramin[®]). The second diet was a mixture of equal parts bovine liver powder and tuna meal with a small addition of vitamin mix and was originally developed by the FAO/IAEA in Seibersdorf for *Anopheles arabiensis* but has also been used in the rearing of *Ae. albopictus* (FAO/IAEA diet). The third diet tested is also a product of the IPCL but was developed specifically for *Ae. albopictus*. It consists of a mixture of tuna meal, bovine liver powder, squid liver powder, brewer's yeast and a small addition of vitamin mix (FAO/IAEA diet II).

During the study, 4% solutions of each diet were tested and all other parameters (larval density, amount of rearing liquid, amount of diet administered) remained constant. Survival from L1 until pupation and until emergence, duration of the immature stage, proportion of males, and size were assessed.

Immature stage duration was significantly affected by the diet used, with both FAO/IAEA diet and FAO/IAEA diet II fed larvae having significantly shorter larval durations than those fed with CAA diet. Pupal periods were also shorter when larvae were fed on FAO/IAEA diet and FAO/IAEA diet II, with significantly higher proportions of the total males emerging within the first 24 hours of pupation.

This is important because during mass-rearing, it is preferable to have high production in a short time. Survivorship and size as measured by wing length did not differ significantly across diet types. Based on these findings, rearing of *Ae. albopictus* with FAO/IAEA diet II has been recommended for further rearing of this species.

Based on these findings, the FAO/IAEA diet II has been recommended for the rearing of *Ae. albopictus* in view that it appears to be 30% less expensive than the original FAO/IAEA diet, as the most expensive component (bovine liver powder) has been partially replaced by the squid liver powder (100 times less expensive).

Reports.

Consultants Meeting on the Role of Bacteria on Nutritional Status and Reproductive Success in Fruit Fly Pests in Support of SIT Application. 7-11 February 2011, Vienna, Austria.

Meeting participants reviewed the rapid progress being made in understanding insect-microorganism interactions, and prepared a proposal for a CRP on the 'Use of symbiotic bacteria to reduce mass-rearing costs and increase mating success in selected fruit pests in support of SIT application'.



Participants of the Consultants Meeting on the Role of Bacteria on Nutritional Status and Reproductive Success in Fruit Fly Pests in Support of SIT Application (Vienna, Austria).

Insects are indisputably the dominant multicellular organisms in terrestrial habitats. As such they maintain intricate and complex interactions with other organisms in their habitat. Some of these interactions, such as those between insects and plants, or insects and vertebrates, have been extensively studied. On the other hand, the associations between insects and microorganisms, while pervasive and of paramount ecological and evolutionary importance, are only gradually being understood. Insects depend on symbiotic associations with a variety of microorganisms, which affect many aspects of host biology and physiology including nutrition, immunity, mating behaviour and reproduction.

The insect symbiotic associations are currently artificially divided into three categories:

- a) The first category includes symbionts that provide nutrients such as amino acids and vitamins to their hosts through mutualistic associations.
- b) The second category includes symbionts that provide their hosts with the ability to survive heat stress, to develop resistance to parasitic wasps and/or microbial pathogens, and to exhibit altered host plant preference.

c) The third category includes symbionts that manipulate the reproductive properties of their hosts, inducing phenomena such as parthenogenesis, feminization, male-killing and cytoplasmic incompatibility (CI), which is a kind of male sterility.

Flies and moth pests cause significant and widespread damage to fruit and vegetable production. Insecticide application remains the predominant method of controlling these pests. Indeed, these high value crops are the main targets of agrochemicals, currently receiving 32% of all applications in agriculture. Despite this growing dependence on agrochemicals, suppression of the pest populations is frequently inadequate. In addition, due to regulation, pest resistance, environmental and human health concerns, there is an increasing demand for the replacement of the intensive use of these chemicals by environmentally friendly, effective and sustainable methods, within integrated management approaches. Chief among these are the sterile insect technique (SIT) and related biological control applications.

The efficacy of the SIT is determined by the quality of insects mass-reared in production facilities, irradiated and released in the field. Constraints on the quality of these insects are manifest at every stage of production and major efforts have been made to ensure quality within reasonable economic limits. Indeed the increased use of the SIT is frequently limited by cost-benefit considerations, as the mass-rearing of target insects, their delivery and release in prime condition may be too expensive in some situations. Accordingly, efforts to streamline the SIT process, combining improved quality of sterile insects with reduced production costs should enable the increased application of this approach.

In the past, efforts to improve sterile insect quality for fruit fly pests have focused on colonization, mass-rearing, quality control and pre-release handling. The objective of the proposed new CRP is to extend these approaches to manipulating the diverse microorganisms associated with the fruit pests targeted in SIT operations to protect fruit and vegetable crops. Symbiotic organisms can be important at all stages of the SIT.

Four key questions related to symbiotic bacteria will be addressed: a) Can symbionts help reduce the cost of production and increase mass-reared sterile insect quality? b) How are they affected by radiation? c) Can they be used as probiotics during the pre-release period to improve sterile insect quality? and d) Can they be used to develop novel pest control tools, complementary to the SIT?

The announcement of the new CRP with detailed information can be seen in this newsletter under Announcements or under http://www-naweb.iaea.org/nafa/ipc/crp/ new-crps-ipc.html.

Sixth Session of the Commission on Phytosanitary Measures, International Plant Protection Convention, FAO. 14-18 March 2011, Rome, Italy

Agreements with five new Contracting Parties to the IPPC entered into force during 2010: Benin, Kazakhstan, Mongolia, Singapore and Tajikistan, bringing total membership to 177 parties.

The Deputy Director General (DDG) of FAO Ms. Tutwiler, welcomed Commission on Phytosanitary Measures (CPM) members to FAO and wished them a productive meeting. She strongly linked the International Plant Protection Organization (IPPC) work with the global challenges to fight hunger and to protect the environment. She noted that some countries had difficulties implementing the International Standard for Phytosanitary Measures (ISPMs) due to a lack of capacity.



Commission on Phytosanitary Measures meeting (FAO Headquarters, Rome, Italy.

The Secretariat of the IPPC thanked the IAEA for hosting and funding the *Technical Panel on Pest Free Areas and Systems Approach for Fruit Flies* (TPFF) meeting held in Vienna, Austria in August 2010, and the support given to the development of post-harvest irradiation treatments, including through participation in the IPPC *Technical Panel on Phytosanitary Treatments* (TPPT). The CPM adopted Appendix 1 of ISPM 26 on *Fruit Fly Trapping*. Three new irradiation treatments (for *Cylas formicarius elegantulus*, for *Euscepes postfaciatus*, and for *Ceratitis capitata*) were also adopted by the CPM, as Annex of ISPM 28 (*Phytosanitary Treatments For Regulated Pest*).

In line with the Member States needs presented during the CPM, the Joint FAO/IAEA Division was requested to continue developing technically and scientifically sound reference manuals, thereby providing its Member States with useful guidance to apply international standards and to conduct certain phytosanitary activities. Additionally, it was requested to continue to actively support capacity building and the implementation of ISPMs in developing countries through regional courses and field projects, in coordination with other international organizations.

Standards Committee Meeting, International Plant Protection Convention, FAO. 2-6 May 2011, Rome, Italy

There were 25 officially nominated members representing FAO's seven regions.

During the meeting, two main issues in relation to the *Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies* (TPFF) were discussed:

- The draft ISPM, '*Protocol to determine host status of fruits and vegetables to fruit fly (Tephritidae) infes-tation*' was discussed. The draft standard was found to be technically sound but needs format input from SC members with experience in drafting standards rather than sending if back to the TPFF for additional review.
- The specification '*Establishment and maintenance of fruit fly quarantine areas within a pest free area in the event of outbreak detection*' was approved by the SC after Member consultation. The specification will be developed as the Annex of ISPM 26 during the next TPFF meeting (22-26 August 2011 in Recife, Brazil).

Announcements

Call for Submission of Research Proposals for a new FAO/IAEA Coordinated Research Project on 'Use of Symbiotic Bacteria to Reduce Mass-Rearing Costs and Increase Mating Success in Selected Fruit Pests in Support of SIT Application'

The sterile insect technique (SIT) is a sustainable and environment friendly control method against some major pests of fruits and vegetables world-wide. One of the main obstacles to the widespread implementation of the SIT is the cost relative to other, less sustainable alternatives.

In recent years there has been a major paradigm shift in understanding the intimate relationship between microorganisms and their hosts, be they humans, animals or plants. Specifically, studies on insects have revealed seminal contributions of microorganisms to the nutrition, health and reproductive success of their hosts. Furthermore, there is evidence that during the mass-rearing and radiation processes, the native microflora of the insects is disrupted and its contribution to the host diminished.

Accordingly, the objective of this Coordinated Research Project (CRP) is to characterize the microorganisms associated with the major fruit pests targeted by the SIT and to harness these symbionts to decrease production costs and increase sterile insect quality. Specifically, the CRP seeks to resolve four key questions related to symbiotic microorganisms:

- 1. Can symbionts help reduce the cost of production and increase mass-reared sterile insect quality? Under natural conditions, fruits used by larvae have extremely low amino acid contents. Ovipositing females inject their eggs into fruit along with bacteria that fix atmospheric nitrogen and others that break down the fruit to produce nutrients essential for larval growth. The mass-rearing process frequently disassociates the reared insects from their native microflora, allowing the proliferation in larval media of opportunistic microorganisms that may not be beneficial. Yeasts to provide nourishment, and chemicals to suppress opportunistic microorganisms, represent by far the largest cost of larval diets. Adding endogenous symbiotic bacteria to the artificial larval diet may significantly:
 - a) reduce mass-rearing costs by eliminating the need for yeasts and chemicals
 - b) prevent the growth of competing microorganisms
 - c) improve mass-rearing efficiency and quality of the insects produced.
- 2. How are they affected by radiation? Evidence suggests that radiation of mass-reared flies can disrupt the symbiotic community by favouring some bacterial

species and suppressing others. Understanding the effects of radiation may enable us to design responses that address them in a manner that optimizes the SIT efficiency. In addition, radiation may also result in the development and isolation of mutant strains of endogenous symbiotic bacteria leading to novel insect symbiotic associations with desirable traits. Such development may eliminate the need of intensive genetic screens and/or the application of transgenic/paratransgenic approaches.

- 3. Can they be used as probiotics during the pre-release period to improve sterile insect quality? In nature symbiotic bacteria become established in the gut of adult flies. These appear to play an important role in the reproductive success of males. The complement of bacteria present in released males following massrearing and irradiation may differ from their wild counterparts enough to impede their performance. There is preliminary evidence that restoring the symbiotic bacteria, prior to release, can significantly improve their sexual performance. In the Mediterranean fruit fly, enriching the sterile insect diet with naturally occurring bacterium Klebsiella oxytoca significantly improved sterile male mating competitiveness in the laboratory and in field cages. In addition, bacterially enriched sterile males inhibited female receptivity to re-mating more efficiently than sugar fed males and survived longer periods of starvation. These results suggest that inoculating mass-reared sterile flies with bacteria prior to their release is a valid approach to improve the efficacy of the SIT. It is worthwhile to validate this approach at an operational level and to extend it to other insects targeted by the SIT.
- 4. Can they be used to develop novel pest control tools, complementary to the SIT? Certain symbiotic bacteria are known to manipulate mating behaviour and reproduction of their hosts. Identifying these organisms and introducing them to target populations can effectively reduce pest populations and their economic impact. For example, the incompatible insect technique (IIT) employs cytoplasmic incompatibility, induced by insect symbionts such as Wolbachia species. In a Wolbachia-based IIT strategy, female sterility is artificially sustained by repeated releases of cytoplasmically incompatible males. Since Wolbachia is not paternally transmitted, the infection type present in the release strain does not become established in the field. Similar to the conventional SIT, the increasing ratio of incompatible matings over time can lead to population suppression. This strategy has been successfully tested under laboratory conditions for two major agricultural pests, the Mediterranean fruit fly and the olive fly. It is worthwile that such an approach be validated and extended, alone and/or in conjuction with the SIT, to other target insect pest species.

The expected duration of the CRP is 5 years (2012-2017) and the first Research Coordination Meeting is planned for April 2012 in Vienna, Austria. Scientists and researchers who are interested in collaborating in this new CRP should contact Rui Cardoso Pereira (R.Cardoso-Pereira@iaea.org).

Information on the IAEA Coordinated Research Programme and how to apply for research contracts and research agreements can be found at http://wwwcrp.iaea.org/. Applications should be submitted by late November 2011.

New FAO/IAEA Guidelines for Implementing Systems Approaches for Pest Risk Management of Fruit Flies

International trade in commodities provides food, consumer goods, and a livelihood to millions of people, but can also spread pests that cause serious damage to commercial crops and to the environment. Many Tephritidae fruit fly species are important plant pests, due to tendencies towards high fecundity, wide host range and potential to cause serious damage. These fruit fly species often are categorized as quarantine pests.

Some of the stand-alone options for managing fruit fly risk are non-host status, pest free areas, and commodity treatments. However, pest risk management measures may be combined in a systems approach as described in the International Standard on Phytosanitary Measures No. 14 (The Use of Integrated Measures in a Systems Approach for Pest Risk Management).

Measures may be applied sequentially in the exporting country at the time of pre-harvest, harvest, post-harvest, export and transport, or at entry and distribution to the importing country. Area-wide integrated pest management programmes against fruit flies can play a significant role in suppressing pest populations to a low level during the pre-harvest time, thereby supporting the efficacy of all subsequent measures applied after harvesting.

Systems approaches have been applied successfully for many years to various combinations of different species of pest, of host and of area. Yet, NPPOs still encounter challenges to their application. The examples and descriptions in these guidelines seek to support their use against fruit fly pests. These guidelines can be found in our webpage, in the following link http://www-naweb. iaea.org/nafa/ipc/public/technical-reports-ipc.html.

Publication of Special Issue of Genetica

This special issue of Genetica on 'Molecular Technologies to Improve the Effectiveness of the Sterile Insect Technique' reviews the state-of-the-art in the use of genetics and molecular biology to generate improved strains for the application in the sterile insect technique (SIT) as one additional tool in an integrated approach to combat pest insects. The special issue, edited by Gerald Franz, represents the outcome of a Coordinated Research Project managed and funded by the Joint FAO/IAEA Division. It contains 15 scientific papers and is available at http:// springerlink.metapress.com/content/0016-6707/139/1/.

The table of contents of Genetica 139 (1) (2011) is:

G. Franz and A.S. Robinson. Molecular technologies to improve the effectiveness of the sterile insect technique.

R.C. Smith and P.W. Atkinson. Mobility properties of the Hermes transposable element in transgenic lines of *Aedes aegypti*.

J.N. Shukla, S. Jadhav, J. Nagaraju. Novel female-specific splice form of *dsx* in the silkworm, *Bombyx mori*.

T. Nolan, P. Papathanos, N. Windbichler, K. Magnunsson, J. Benton, F. Catteruccia, A. Crisanti. Developing transgenic *Anopheles* mosquitoes for the sterile insect technique.

F. Scolari, P. Siciliano, P. Gabrieli, L.M. Gomulski, A. Bonomi, G. Gasperi, A.R. Malacrida. Safe and fit genetically modified insects for pest control: from lab to field applications.

J.S. Mesa, X. Nirmala, G.J. Zimowska, C.S. Zepeda-Cisneros, A.M. Handler. Development of transgenic strains for the biological control of the Mexican fruit fly, *Anastrepha ludens*.

M.J. Scott, A. Atapattu, A.H. Schiemann, C. Concha, R. Henry, B.L. Carey, E.J. Belikoff, J.C. Heinrich, and A. Sarkar. Organisation and expression of a cluster of yolk protein genes in the Australian sheep blowfly, *Lucilia cuprina*.

M.F. Schetelig, F. Götschel, I. Viktorinová, A.M. Handler, E.A. Wimmer. Recombination technologies for enhanced transgene stability in bioengineered insects.

A. Zacharopoulou, A.A. Augustinos, W.A.A. Sayed, A.S. Robinson, G. Franz. Mitotic and polytene chromosomes analysis of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae).

K.A. Raphael, D.C.A. Shearman, K. Streamer, J.L. Morrow, A.M. Handler, M. Frommer. Germ-line transformation of the Queensland fruit fly, *Bactrocera tryoni*, using a *piggyBac* vector in the presence of endogenous *piggyBac* elements.

G. Saccone, M. Salvemini, L.C. Polito. The *transformer* gene of *Ceratitis capitata*: a paradigm for a conserved epigenetic master regulator of sex determination in insects.

R. Permpoon, N. Aketarawong, S. Thanaphum. Isolation and characterization of *Doublesex* homologues in the *Bactrocera* species: *B. dorsalis* (Hendel) and *B. correcta* (Bezzi) and their putative promoter regulatory regions.

N. Aketarawong, S. Chinvinijkul, W. Orankanok, C.R. Guglielmino, G. Franz, A.R. Malacrida, S. Thanaphum. The utility of microsatellite DNA markers for the evaluation of area-wide integrated pest management using SIT

for the fruit fly, *Bactrocera dorsalis* (Hendel), control programs in Thailand.

K.P. Arunkumar, J. Nagaraju. Drosophila *intersex* orthologue in the silkworm, *Bombyx mori* and related species.

M. Wu, Z. Sun, G. Luo, C. Hu, W. Zhang, Z. Han. Cloning and characterization of *piggyBac*- like elements in lepidopteran insects.

Rearing Codling Moth for the Sterile Insect Technique

Over the past years, there has been an increasing interest to develop the sterile insect technique (SIT) for the codling moth Cydia pomonella for integration with other control tactics. The codling moth is amongst the most severe pests of pome fruit in the temperate regions of the world. Broad-spectrum insecticides have mainly been used to control this pest resulting in several negative environmental consequences. The demand for alternative control techniques is therefore increasing worldwide, and includes synthetic growth regulators, mating disruption, attract and kill, microbiological control agents, and the sterile insect technique. The integration of sterile insects with these control practices within the context of areawide integrated pest management offers great potential as shown by the on-going SIR Programme in British Columbia, Canada. However, efficient and effective massrearing of the target insect is a fundamental component of the SIT but its complexity for lepidopteran pests is very often under-estimated.

A new FAO/IAEA book has been published in the Plant Production and Protection Paper series of the Food and Agriculture Organization of the United Nations. The book compiles and summarizes all available information on the rearing of the codling moth in relation to the SIT. Aspects such as colonization, adult and larval diet, sexing, quality control, shipment, disease control, data recording and management are described.

The book is not written to be read from cover to cover, but the information is presented so that individual sections can be consulted by the reader when necessary. The document therefore, does not provide guidelines *per se*, nor is a compendium of standard operating procedures, as these will need to be developed for each rearing facility based upon local needs and availability of materials and ingredients.

The book was authored by Dr V.A. Dyck, a former staff member of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The book contains 197 pages and is illustrated with 32 figures and 14 tables. For more information, please visit http://wwwnaweb.iaea.org/nafa/ ipc/public/ipc-codling-moth-sterileinsect-technique-2010.pdf where the PDF of the book can be downloaded.

Announcement of FAO/IAEA Regional Training Courses

Standardized Entomological Monitoring, Data Collection and GIS-Aided Data Processing as Needed for Area-Wide Integrated Pest Management Campaigns Against the Tsetse and Trypanosomosis Problem (under TC Project RAF5060). 14 November - 2 December 2011, Bobo-Dioulasso, Burkina Faso.

Application procedure: Nominations should be submitted on the standard IAEA application form for training courses/workshops (downloadable from: http://www-tc.iaea.org/tcweb/participion/astrainee/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, or the office of the FAO Resident Representative or the Ministry of Agriculture).

The completed forms must be received by 15 August 2011 at the International Atomic Energy Agency, Vienna International Centre, P.O. Box 100, 1400 Vienna, Austria. Advance nominations by facsimile (+43-1-26007) or e-mail (official.mail @iaea. org) are welcome.

Standardized Collection and Processing of Tsetse Flies for Molecular Tsetse Population Genetic and Morphometric Analyses (under TC Project RAF5060). 21 November - 5 December 2011, Muguga-Nairobi, Kenya.

Application procedure: Same as above, but with the deadline for nominations, 22 August 2011.

Molecular Biology Training Opportunities

The tsetse group at the Insect Pest Control Laboratory in Seibersdorf has limited places for training fellows in molecular techniques related to our work on the *Glossina pallidipes* Salivary Gland Hypertrophy Virus. These places would be suitable for recent graduates at MSc or PhD level with experience of molecular techniques who would like to gain further experience whilst contributing to our research programme. Places would be for 6–12 months and are subject to the availability of funds under the TC programme.

Prospective candidates with the necessary qualifications from tsetse affected Member States are encouraged to apply on the IAEA standard TC fellowship form (http://tc.iaea.org/tcweb/default.asp) through their National Liaison Officer. For informal enquiries contact the laboratory head Marc Vreysen (<u>M.Vreysen@iaea.org</u>).

Other News

Pesticide Use Drops Thanks to SIR Program in British Columbia, Canada

There's been a 95 per cent reduction in the amount of organo-phosphate pesticides used in the Okanagan Valley in the past 19 years due to the sterile insect release (SIR) program used to combat codling moth in apple orchards. Entomologist Hugh Philip was reporting to the SIR board in Kelowna, which is made up of representatives from the four regional districts that are part of the area-wide program. "The program has had a considerable impact on the prevalence of codling moth," reported Philip, and that has translated into far fewer pesticides being applied in orchards.

It's still necessary for people with backyard host trees such as apple, pear and crabapple to control codling moth or else strip all the fruit from their trees while it's still immature. Otherwise, commercial orchards could be re-infested with codling moth.

In areas around orchards, this year 240 residential property-owners have been ordered by the SIR program to strip the fruit from their trees, while eight commercial orchards have also been ordered to strip their fruit because of a high level of infestation of codling moth, which could re-infest their neighbours' fruit.

Orchardist Fred King of Kelowna, who is also a member of the SIR board and of the B.C. Fruit Growers' Association board, commented that there are still some orchardists who automatically put on three cover sprays a year for codling moth, whether it's needed or not. "We have to change that," he commented.

Philip said few orchardists should need to spray even once this year for codling moth.

Organic orchardist Brian Mennell from Cawston noted that by reducing sprays, orchardists would find that they would be permitting the survival of beneficial insects, some of which would help to keep pests in check without chemicals.

Board member and orchardist Allan Patton said he stopped spraying 12 years ago and found a bit of a jump in damage from other pests for the first couple of years, but then those problems dropped right off and he hasn't sprayed since.

Source: Kelowna Capital News (kelownacapnews.com, 22 May 2011).

Bilateral Collaboration between SlovakAid and Kenya Against the Tsetse and Trypanosomosis Problem

Over the last ten years the Institute of Zoology, Slovak Academy of Sciences (IZ SAS) in Bratislava, Slovakia, has been collaborating with the FAO/IAEA in research and methods development for addressing the tsetse and trypanosomosis problem in Africa and in maintaining back-up or 'seed' colonies of three important tsetse fly species in support of on-going and planned tsetse sterile insect technique (SIT) programmes in Africa. The IZ-SAS also assists FAO and IAEA and their Member States in providing training for scientists and technicians from collaborating African institutions on tsetse mass rearing and aspects relevant to developing and applying SIT. This fruitful collaboration between IAEA, FAO and the IZ SAS was instrumental in engaging the SlovakAid to provide bilateral technical assistance amounting to € 174 000 to the Trypanosomosis Research Centre of the Kenya Agriculture Research Institute (KARI-TRC) in Muguga, Nairobi, Kenya.



Commissioning Ceremony on 8 December 2010: Ms Florence Wamwiri explaining activities under the collaborative effort to high level representatives of Kenya and Slovakia.

The SlovakAid support was used to refurbish, equip and upgrade the KARI-TRC tsetse rearing facility and to assist in tsetse research and control activities. Furthermore, support was provided to the KARI-TRC molecular genetics laboratory, which is among the leading institutions in Africa for research on tsetse population genetics. During the first year of the collaboration (2010), the KARI-TRC insectaries were equipped with the newly developed tsetse production unit (TPU-4), which now enables the Centre to increase its tsetse rearing capacity to about 100 000 colony female flies. Two KARI-TRC staff obtained two-month SlovakAidsponsored fellowship training at the IZ SAS. The official commissioning of the newly equipped facilities at the KARI-TRC took place on December 8, 2010, in the presence of Kenyan officials, the Ambassador of Slovakia Republic and representatives of the IZ-SAS. Activities under the bilateral cooperative effort in 2011 focus on increasing the Glossina pallidipes colony at KARI-TRC and generating progress towards the initiation of pilot SIT releases in the Lambwe Valley, Kenya.

Source: Milan Kozanek, Director of Institute of Zoology, Slovak Academy of Sciences.



Easter Island map showing fruit fly detection system and Bactrocera dorsalis outbreak area (Courtesy of Chile National Fruit Fly Programme).

Oriental Fruit Fly Eradicated from Easter Island, Chile

In December 2010, the agricultural authorities (SAG, Servicio Agrícola y Ganadero) of Easter Island (Rapa Nui) in Chile detected in one of the detection traps an insect specimen that appeared to be an Oriental fruit fly (*Bactrocera dorsalis*). The specimen was identified and confirmed as Oriental fruit fly, and an additional couple of specimens were detected later that same month.

The response of the National Fruit Fly Programme (Programa Moscas de la Fruta) was immediate. In less than 2 weeks, staff (including a taxonomist and temporary employees) and materials (identification materials, vehicles, insecticides, traps, etc...) were sent to the island to initiate the eradication campaign.

The first measure was the distribution of 232 additional traps baited with malathion and methyl eugenol (ME) or traps with protein in the vicinity of the initial captures. The traps were serviced twice a week to monitor the outbreak area. Additionally, 670 kg of host fruits were inspected for the presence of larvae, 3 698 kg of

host fruits were destroyed and 27 431 m² of soil was treated and 3 500 L of spinosad applied. Additionally strict quarantine measures were implemented to guarantee that any fruits sent to mainland Chile were fruit fly free.



The outbreak most likely originated from Tahiti, where the pest is present and from where a weekly flight connection with Easter Island exists. Additionally some cruises and small boats arrive at the island.

Quarantine inspection with X ray machines is always in place at the port of entry, however the infested fruit originating the outbreak was not detected at a port of entry. The same occurred in 1974, but that time with *Bactrocera tryoni*, that was successfully eradicated.

Finally, on 27 April 2011 the outbreak was declared eradicated. The total cost of the campaign was approximately US \$100 000.

Source: Ricardo Rodríguez, Head of the Chile National Fruit Fly Programme (May 2011).

Two Areas Comprising 299 000 Hectares Are Declared Mediterranean Fruit Fly Free in Guatemala

The Guatemalan Minister of Agriculture (MAGA) has declared as Mediterranean fruit fly free Los Huistas, a region comprising 228 000 hectares (2 280 km²) located in the northwestern border with Mexico. In Salcaja, amid the country's central plateau another 71 000 hectares (710 km²) have also been declared free of economically important species of fruit flies (see map below, courtesy Programa Moscamed, Guatemala). These achievements are part of a continued effort of the trilateral Mediterranean Fruit Fly Programme which includes the governments of Guatemala, Mexico and USA, technically backed by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the IAEA's Department of Technical Cooperation.



The Mediterranean fruit fly was eradicated from these areas integrating sterile males of the Vienna-7 genetic

sexing strain developed at the FAO/IAEA Agriculture and Biotechnology Laboratories, and produced in the world's largest insect mass rearing facility at El Pino, Guatemala.

Eradication activities were outlined and supported by an international technical advisory panel including staff of the Insect Pest Control Section. The initiative to develop these areas as free of the Mediterranean fruit fly was also supported through two IAEA's technical cooperation projects.



The government of Guatemala issued the official declaration of eradication in the Los Huistas Region on 6 January 2011, and in Salcaja on 19 April 2011, according to the requirements of the international phytosanitary framework established by the FAO's International Plant Protection Convention (IPPC) and the WTO's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement).

Based on this achievement Guatemala has started negotiations with commercial partners to certify Los Huistas and Salcaja as Mediterranean fruit fly free to be able to export fresh fruit and vegetables grown in this region.

Source: Programa Moscamed, Guatemala (May 2011).

Panama Aims to Become Sterile Fruit Fly Producer

Panama could become a center of production and distribution of sterile Mediterranean fruit flies to help eradicate the pest, which is one of the most destructive in agriculture, according to local news website Prensa.com.

Agricultural authorities in Panama are negotiating with the United States to produce sterile flies in a plant that already produces sterile males to fight another type of pest, the New World screwworm, that attacks cattle and other livestock, said Emilio Kieswetter, Minister of Agricultural Development, according to the website. The plant is a joint project of Panama and the USA.

"Once obtaining the approval of the U.S., an annex to the plant would be built and the same mechanism that has been developed for the eradication of the livestock pest (screwworm fly) would be implemented," Kieswetter is quoted as saying. The Mediterranean fruit fly attacks mature fruit, pushing its ovipositor inside the skin and depositing its eggs in the perforation. The eggs become larvae that then eat the fruit pulp. The fly particularly affects citrus, guava, papaya, coffee, tomatoes and star apples in Panama, the website said.



In an eradication program, the sterile male flies are released into the field, where wild females mate with them but do not produce fertile eggs.

Source: www.freshfruitportal.com (31 January 2011).

Surveillance and Monitoring for the Invasive Fruit Fly (*Bactrocera invadens*)

Bactrocera invadens, is a pest of Asian origin capable of infesting many commercial fruit crops. It was first detected on the African continent, in Kenya, in 2003. It is now listed in most countries in Western, Eastern and Central Africa as well as in some Southern African countries: Mozambique, Zambia and Namibia. In 2010, specimens of this invasive pest were intercepted in South Africa, in two separate areas in northern Limpopo, and in Botswana's Chobe district.

In South Africa, early detection of the pest allowed for timely intervention in both areas where *B. invadens* was intercepted. The pest has been successfully eradicated from South Africa. Eradication was achieved through early detection of the incursion and the rapid deployment of ground-applied male annihilation treatments and air/ground-applied protein bait treatments. Interceptions of this fly in South Africa and Botswana last year are an indication that the areas in Southern Africa currently free of this pest are under risk of incursions. In accordance with the recommendations of the National B. invadens Steering Committee of South Africa, all producers of export fruit are called upon to commence monitoring for *B. invadens*. It is especially important for all growers producing fruit for export to commence with on-farm monitoring and to maintain such monitoring as evidence that the farm remains free of this pest.

Source: National B. invadens Steering Committee of South Africa (28 March 2011).

Phytosanitizing Hawaiian Fruit: A Technology Transfer Success Story

Papaya, rambutan, longan, dragon fruit, and purplefleshed sweetpotato are just some of the delicious tropical fruits and vegetables gaining popularity in the continental USA. Chances are these delectable delights, now found in grocery stores and specialty Asian supermarkets all over the country, were grown in Hawaii.

But just 5 years ago, one would have been hard pressed to find these healthy and tasty Hawaiian treats in the continental USA. That is because the export potential of Hawaiian produce was limited by strict quarantine restrictions and phytosanitary measures to ensure that agricultural pests such as fruit flies didn't invade the mainland. These export restrictions have cost Hawaiian growers around US \$300 million per year in lost sales.

Research by entomologist Peter Follett and food technologist Marisa Wall has changed all of that. The scientists, who work at the Pacific Basin Agricultural Research Center in Hilo, Hawaii, are the first to apply generic irradiation protocols to control a wide variety of quarantine insect pests found on fresh commodities.



Biological science technician Sandra Silva analyzes peel color of irradiated dragon fruit as part of the quality analysis of irradiated tropical crops.

Based partly on the scientists' extensive research, the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) in 2006 published a landmark rule accepting the generic doses for treatment of Hawaiian produce. "APHIS had the courage to take the step of turning our research into regulations. Without their hard work, growers and consumers wouldn't be able to benefit from this research," says Follett.

But just because a generic dose is available doesn't mean growers will use it. They want to ensure that their product will still be at its best when it reaches the mainland. That's where Wall comes in. She's responsible for examining product quality after exposure to radiation.

"Irradiation adds another step to the postharvest process, which puts added stress on the commodity," says Wall. "To establish maximum dose levels, we conducted tests for composition, quality, and visual damage to see exactly how much radiation the product can tolerate. We also replicated shipping and storage conditions to assess whether the consumer would receive a highquality product."



Slices of fresh dragon fruit, revealing its edible flesh.

Wall concluded that most commodities can tolerate irradiation at levels that control pests. But she cautions that not all commodities behave the same when exposed to radiation. "We found that different varieties of a fruit or vegetable react differently to the same radiation doses. Maturity, time of harvest, and several other factors can also affect product quality."

Variations aside, Follett and Wall's research has opened up the market for Hawaiian produce. The Island State currently uses generic irradiation treatments to export 15-20 million pounds of various tropical fruits and vegetables annually.

The technology has made it easier and less costly for Hawaiian growers to share their produce with consumers on the mainland. As a result of their efforts, Follett and Wall received a 2010 Federal Laboratory Consortium Award for Excellence in Technology Transfer.

Hawaiian growers and exporters are not the only ones benefitting from the scientists' research. In 2009, the International Plant Protection Convention approved the generic radiation dose of 150 Gy for tephritid fruit flies, facilitating the worldwide adoption of this technology. There are currently a handful of countries using the generic protocols on a variety of commodities. India, Thailand, Vietnam, Mexico, and Pakistan recently received APHIS approvals to export tropical fruits to the USA using generic irradiation treatments. Indonesia, the Philippines, Peru, and South Africa are awaiting their approvals.

Source: USDA-ARS http://www.ars.usda.gov/is/AR/archive/feb11/ fruit0211. (February 2011).

Special Recognition on UN Women's Day in Sudan

The IAEA is supporting the Government of Sudan with its efforts to develop the sterile insect technique for the malaria transmitting mosquito *Anopheles arabiensis* and to assess in a pilot area in the Northen State whether the SIT can be integrated as part of area-wide integrated pest management strategies against this vector.



Special recognition for Badria Babiker El Sayed.

The counterpart of this project, Badria Babiker El Sayed of the Tropical Medicine Research Institute in Khartoum, together with only 9 other women selected from various disciplines in the entire country, received special recognition on UN Women's Day from H.E. Omer Hassan Al-Bashir, the President of Sudan, for her outstanding professional achievements.

Source: Badria Babiker El Sayed, Tropical Medicine Research Institute in Khartoum.

Interesting Published Articles

Phytosanitary applications of irradiation

G.J. Hallman

USDA-ARS, Weslaco, TX, USA

Abstract

Phytosanitary treatments are used to disinfest agricultural commodities of quarantine pests so that the commodities can be shipped out of quarantined areas. Ionizing irradiation is a promising phytosanitary treatment that is increasing in use worldwide. Almost 19 000 metric tons of sweet potatoes and several fruits plus a small amount of curry leaf are irradiated each year in 6 countries, including the United States, to control a number of plant quarantine pests.

Advantages over other treatments include tolerance by most fresh commodities, ability to treat in the final packaging and in pallet loads, and absence of pesticide residues. Disadvantages include lack of acceptance by the organic food industries and logistical bottlenecks resulting from current limited availability of the technology.

A regulatory disadvantage is lack of an independent verification of treatment efficacy because pests may be found alive during commodity inspection, although they will not complete development or reproduce. For phytosanitary treatments besides irradiation, the pests die shortly after the treatment is concluded. This disadvantage does not hamper its use by industry, but rather makes the treatment more difficult to develop and regulate.

Challenges to increase the use of phytosanitary irradiation (PI) are cost, because commercial use has not yet reached an optimum economy of scale, lack of facilities, because of their cost and current inability to feasibly locate them in packing facilities, lack of approved treatments for some quarantine pests, and concern about the process by key decision makers, such as packers, shippers, and retailers. Methods for overcoming these challenges are discussed.

The full paper was published in: Comprehensive Reviews in Food Science and Food Safety (2011) 10:143-151.

Genetic structure and demographic history of new world screwworm across its current geographic range

P. Fresia¹, M.L. Lyra¹; A. Coronado², and A.M.L. Azeredo-Espin^{1,3}

¹ Laboratório de Genética e Evolução Animal-Centro de Biologia Molecular e Engenharia Genética and Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo,Brazil

² Area de Parasitología Veterinaria, Universidad Centroccidental "Lisandro Alvarado", Barquisimeto, Lara, Venezuela

³ Departamento de Genética e Evolução e Bioagentes, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

Abstract

The phylogeographical history of the pest fly screwworm, *Cochliomyia hominivorax* (Coquerel), was studied using partial mitochondrial DNA sequences of the control region, Cytochrome c oxidase (CO) subunit I and CO subunit II from 361 individuals collected across its current geographic range. Analyses showed marked genetic differentiation on a macrogeographic scale.

The genetic diversity in the species is structured into four main 'regional groups,' corresponding to Cuba, the Dominican Republic, and the North and South Amazon region. Results indicated that the distribution of screwworm genetic diversity was mainly shaped by historical events, i.e. colonization of Caribbean islands, vicariance in the Amazon region and population expansion.

Demographic history analyses revealed that the population expansion started 20-25,000 yr ago and recently increased exponentially. We hypothesized that the initial period of expansion was probably associated with environmental amelioration in the late Pleistocene and the exponential increase with resource availability in recent times. The population expansion is probably responsible for the low divergence and the lack of genetic and geographic correlation in the South Amazon region but did not erase the genetic structure pattern on a continental scale.

The screwworm is one of the most damaging livestock pests in South and Central America, and the pattern of genetic variability distribution reported here suggests that the Caribbean area and the North and South Amazon regions could be considered as independent units for future pest control programs.

The full paper was published in: Journal of Medical Entomology (2011) 48:280-290.

Katie Vicari

No refuge for insect pests

K. Wu

State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China.

The sterile insect technique offers an alternative to the refuge strategy for managing resistance to *Bt* toxins (from Nature Biotechnology (2010) 28:1273-1275).



Figure 1 Use of the SIT together with transgenic cotton expressing the *Bt* transgene suppresses the growth of the pink bollworm population and facilitates management of resistance to *Bt* toxin. Pink bollworm feeds only on cotton bolls and does not damage other tissues. (a) Sustainable use of *Bt* cotton to control pink bollworm populations is threatened by the emergence of resistance. (b) Although costly, repeated release of sterile pink bollworm moths (red) in vast excess to the number of wild moths (brown) can suppress the growth of pink bollworm populations. (c) Combined use of *Bt* cotton and SIT ensures that the release of fewer sterile moths can suppress the growth of pink bollworm populations while preventing the emergence of resistance to *Bt* toxin.

The full paper was published in: Nature Biotechnology (2010) 28:1273-1275.

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ABD-ALLA, A.M.M., A.G. PARKER, M.J.B. VREY-SEN, and M. BERGOIN. Tsetse salivary gland hypertrophy virus: hope or hindrance for tsetse control. PLOS Neglected Tropical Diseases (in press).

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Insect Pest Control Newsletter, No.77, July 2011

Insect Pest Control Newsletter, No.77, July 2011

Impressum

Insect Pest Control Newsletter No. 77

The IPC Newsletter is prepared twice per year by the Insect Pest Control Subprogramme Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture.

International Atomic Energy Agency Vienna International Centre, PO Box 100, 1400 Vienna, Austria Printed by the IAEA in Austria, Jwy 2011.