

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

# Insect Pest Control Newsletter

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Damage to the *Opuntia* cactus by the Cactus Moth (*Cactoblastis cactorum*), an exotic invasive insect pest species. Inset: *C. cactorum* laying an egg stick.

### A. To the Reader

The year 2003 has again been a very intense period for all of us working at the Insect Pest Control Sub-programme of the Joint FAO/IAEA Agriculture Programme. As you can see from the content of this newsletter, there are many interesting things to report in terms R&D, normative activities, and the application of area-wide control and SIT.

I would like to highlight a number of normative activities in which we are increasingly involved. One that stands out during 2003 is the recent publication of "Trapping Guidelines for Area-wide Fruit Fly Programmes," which responds to the request by Member States to harmonize internationally trapping procedures for Tephritid fruit flies of economic importance. These pest insects have a major impact on the international trade of fresh fruits and vegetables, and the guidelines provide strategic guidance and direction to NPPOs, RPPOs and industry on where and how to implement fruit fly surveys. Using these guidelines in the implementation of surveys will support FAO and IAEA Member States in obtaining international recognition of their fruit fly control and guarantine activities. Other normative activities, which continue to expand, are DIR-SIT, the international directory of SIT facilities and food irradiation facilities and IDIDAS, the international database on insect disinfestations and sterilization http://wwwididas.iaea.org/IDIDAS/start.htm The number of users interacting each month with these web-based information sources is very significant and they have recently been nominated to compete for the annual FAO Agriculture Department prize in the category of databases and information networks. IDIDAS was also included as a reference in the recently approved IPPC standard on food irradiation (International Standards for Phytosanitary Measures No. 18, FAO. 2003).

A new project is a world-directory of fruit fly workers. A tremendous amount of information is made available each year on Tephritid fruit flies: new technologies developed, new information on their biology and ecology; new control methods made available, new species identified, new outbreaks recorded and new operational control programmes launched. This site will attempt to collate this information and allow Tephritid fruit fly workers worldwide to keep up-to-date on the most recent developments.

The database will include powerful search engines and will be available on the Internet. Registration is possible electronically through the web site (*www.tephritid.org*). In order to launch this service, your contribution is needed and we greatly appreciate if you could share this information with colleagues you know work in the fruit fly field. With your help, we are convinced that this database will quickly become an essential tool for Tephritid fruit fly workers worldwide.

Another activity has been the development of more scientific methods for determining when an area achieves a pest-free status. A consultants meeting focused on this topic and a generic procedure has been developed for declaring an area to be "pest-free" following an eradication campaign against an insect pest. This involves a probability model to deal with null trapping results and also a growth model to help verify that pest specimen were not present when control was stopped. Two probability models are developed for a situation in which trapping is proceeding for an insect pest and in which the trapping results are all negative. The models calculate the probability of such negative results if in fact there were insects present. If this probability is sufficiently low, then the hypothesis that insects are present is rejected. The model depends on knowledge of the efficiency of the traps and also the area of attractiveness of the traps. The question is considered of a rebound of an incipient but non-detectable population remaining after control measures are discontinued. Using a growth model, the rate of increase of an insect population starting from one or two insects is examined. In addition, for insects that vector disease, it is suggested that the progress of the vectored disease be monitored to detect continued transmission; this would best be done in conjunction with a disease transmission model.

Other normative and promotional activities under development include guidelines for the design and equipping of fruit fly mass rearing facilities, a book and video on the cactus moth, *Cactoblastis cactorum*, various standard operational procedure manuals, and the first textbook on the SIT. This last one is a major project, under development for some years, that does not review SIT projects, but rather addresses all SIT components in a generic way. The draft will be completed in 2004 for publication in collaboration with Kluwer.

Moving on to R&D activities, the six-year Coordinated Research Project (CRP) "Genetics Application to Improve the SIT for Tsetse Control/Eradication" was concluded and the results will be published as a collection of articles in the Journal Genome. Knowledge of tsetse fly genetics has increased dramatically in these past six years. Three major impacts can be identified: (a) the development of

genetic tools for studying tsetse flies; (b) the development of tsetse as a model system for studies on insect immunity, and (c) the finding that tsetse populations show an unexpectedly high degree of genetic differentiation. All impacts are noteworthy from a purely scientific point of view, and each has profound consequences for the successful application of the SIT.

We have also had a successful start of the CRP on "Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application" with thirteen scientists from ten countries participating. During the 1<sup>st</sup> RCM held in Montpellier, France, participants agreed to focus research during the next six years of this CRP on three major areas of quality control related to a) tsetse diet, b) tsetse rearing, and c) tsetse behaviour.

I would like to call your attention to a new Coordinate Research Project (CRP) on "Improving Sterile Male Performance in Fruit Fly SIT Programmes" that was recommended by a Consultants Meeting and has now been approved for the period (2004-2009). Recent research involving hormonal, nutritional and semiochemical manipulation of emerged sterile flies, to be applied at the emergence / release facilities, has identified significant opportunities to increase sterile male performance to ultimately reduce the cost and increase the effectiveness of SIT programmes. We are encouraging relevant applications for this new CRP. The selection of contract and agreement holders will be based on a good balance between fruit fly action programme staff and scientists conducting basic research in physiology, chemical ecology, microbiology and behaviour.

On behalf of all of us at the Subprogramme, I would like to thank all those of you who are collaborating with us in one way or another for your support and hard work. We look forward to another fruitful year and wish you a very successful 2004.

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Jorge Hendrichs Head, Insect Pest Control Section



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C. FORTHCOMING EVENTS

### I. Research Co-ordination Meetings (RCMs)

- 1. *New* CRP on "Molecular Technologies to Improve The Effectiveness of SIT" 19-23 January 2004, Vienna, Austria. 1<sup>st</sup> RCM.
- "Improvement of codling moth SIT to facilitate expansion of field application" 8-12 March 2004, Stellenbosch, South Africa. 2<sup>nd</sup> RCM.
- 3. "Development of improved attractants and their integration into fruit fly SIT management programmes" (in conjunction with Western Hemisphere Meeting on

### **II.** Consultants and Other Planning Meetings

- Workshop on "Technical and Environmental Feasibility of Sequential Aerosol Treatment for Tsetse Suppression" 1-5 March 2004, Vienna, Austria.
- "Radiation biology studies to optimize sterilization procedures in SIT Programmes" 19-23 April 2004, Vienna, Austria.
- 3. CRP on "Mass production and pre-release biology of *Anopheles arabiensis*" 22-27 March 2004, Vienna, Austria.

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

1. Workshop on "Integration of SIT and Other Environment Friendly Methods for the Management of Key Citrus Insect Pests" 17 February 2004, Agadir, Morocco, in conjunction with the 10<sup>th</sup> International Citrus Congress (15-20 February 2004). Fruit Flies of Economic Importance), 11-15 May 2004, Fort Lauderdale, Florida, USA. 3<sup>rd</sup> RCM.

- "Quality assurance of mass produced and released fruit flies" 18-22 October 2004, Metapa de Dominguez, Chiapas, Mexico. Final RCM.
- 5. *New* CRP on "Improving sterile male performance in fruit fly SIT programmes" 25-29 October 2004, Guatemala City, Guatemala. 1<sup>st</sup> RCM.
- "Guidelines for emergence, packing and release of sterile flies used in areawide suppression/ eradication programmes" 11-15 May 2004, Fort Lauderdale, Florida, USA.
- "Generic design and technical guidelines for the location of tsetse mass rearing facilities" 14-18 June 2004, Vienna, Austria.

 Fifth Meeting of the National Coordinators of medfly SIT projects in the Near East, 18-19 February 2004, Agadir, Morocco.

- 3. Regional Workshop on "Using the Male Annihilation Technique to Control Tephritid Fruit Flies" Reduit, Mauritius, February 2004.
- 4. Fifth Meeting of the Working Group on Fruit Flies of the Western Hemisphere, 16-21 May 2004, Ft. Lauderdale, Florida.
- Workshop on "Areawide Application of the Sterile Insect Technique (SIT)" will be held in conjunction with the 15<sup>th</sup> International Plant Protection Congress in Beijing, China (11-16 May 2004).

6. FAO/IAEA Interregional Training Course on "The Use of the Sterile Insect and Related Techniques for the Integrated Areawide Management of Insect Pests" 4 May - 8 June 2004, University of Florida, Gainesville, Florida, USA.



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### I. Research Co-ordination Meetings (RCMs)

- "Application of genetics to improve the SIT for tsetse" 23-27 June 2003, Edmonton, Alberta, Canada. 4<sup>th</sup> and final RCM.
- "Quality assurance of mass produced and released fruit flies" 19-23 May 2003, Perth, Australia. 3<sup>rd</sup> RCM.
- 3. "Improved and harmonized quality control for expanded tsetse production, sterilization and field application" (in conjunction with IOBC meeting on QA of Mass

### **II.** Consultants and Other Planning Meetings

- 1. Third semi-annual meeting of the USAID-MERC Management Committee for the SIT projects in the Near East" 3-4 February 2003, Vienna, Austria.
- Third coordination meeting on the Central America Fruit Fly Regional Project RLA5045, Guatemala City, 29-30 April 2003.
- Consultants meeting on "Codling moth genetic sexing" 5-9 May 2003, Vienna, Austria.
- 4. Fourth Meeting of the National Coordinators of medfly SIT projects in the Near East, 20-23 May 2003, Vienna, Austria.
- 5. Meeting of representatives of Israel, Jordan and the Palestinian Territories to define and to draft regional project proposals for the

Reared Arthropods), 15-19 September 2003, Montpellier, France. 1<sup>st</sup> RCM.

- "Enabling technologies for the expansion of SIT for old and new world screwworm" 22-26 September 2003, Kluang, Malaysia. 2<sup>nd</sup> RCM.
- "Use of nuclear techniques for the colonization and production of natural enemies" 3-7 November 2003, Vienna, Austria. 3<sup>rd</sup> RCM.

control of Tephritid fruit flies in the Near East, using the Sterile Insect Technique, 26-29 May 2003, Vienna, Austria.

- Meeting on technical and management issues of the TC tsetse project MLI/5/017 "Integrated Control of Animal Trypanosomosis Through the Creation of a Tsetse Fly Free Zone" with participation from Mali, Burkina Faso, PATTEC and FAO/IAEA, 12-16 May 2003, Vienna, Austria.
- Consultants meeting on "Codling moth genetic sexing" 5-9 May 2003, Vienna, Austria.
- 8. GIS and data collection strategy planning workshop for *A. arabiensis* in Northern Sudan, 18-20 June 2003, Vienna, Austria.

- 9. Consultants meeting on "Development of guidelines for verification of tsetse fly free areas and the Trypanosomosis Problem" August 2003, Vienna, Austria.
- 10. Fourth semi-annual meeting of the USAID-MERC Management Committee for the SIT projects in

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

- Postgraduate Course On Codling Moth SIT in Neuquen, Argentina, 10-14 March 2003. Organized by The University of Comahue.
- Fruit fly regional workshop in Hanoi Viet Nam, 12-14 March 2003, organized by Griffith University, Queensland, Australia.
- International Fruit Fly Workshop in Buenos Aires Argentina, 24-26 March 2003.
- 4. FAO Workshop on "Moving from criteria for selection of priority areas to formulation of tsetse and trypanosomiasis field programme proposals: Ethiopia Southern Rift valley-Case Study" Rome, Italy 2-4 July 2003.
- FAO/IAEA Regional workshop on "Tephritid Fruit Fly Identification" 4-8 August 2003, Vienna/Seibersdorf, Austria.
- 6. Twenty-fifth Anniversary of the Moscamed and Tenth Anniversary of the Moscafrut Mass Rearing and Sterilization Facilities.
  9 September 2003, Metapa de Dominguez Chiapas, Mexico.
- Fifteenth International Training Course on Fruit Flies. International Fruit Fly Training Center, Moscamed Programme, 8-26 September, 2003, Metapa de

the Near East" 22-23 September 2003, Vienna, Austria.

 Consultants meeting on "Improving Sterile Male Performance in Fruit Fly SIT Programmes" 20-24 October 2003, Vienna, Austria.

Dominguez Chiapas, Mexico.

- 8. Tenth workshop of the IOBC global working group on "Arthropod mass rearing and quality control" 21-24 September 2003, Montpellier, France.
- Twenty seventh International Scientific Council for Trypanosomiasis Research and Control (ISCTRC),
   29 September-3 October 2003, Pretoria, South Africa.
- 10. IAEA/USDA Training Course on "Cactus moth, *Cactoblastis cactorum*, Surveillance and Control" 17-21 November 2003, Tallahassee, Florida.
- Evaluation and planning meeting on "Transfer of Genetic Sexing Mass Rearing Technologies for Mexican Fruit Fly Production" 4-5 December 2003, Metapa de Dominguez Chiapas, Mexico.
- 12. USDA Cactus moth, *Cactoblastis cactorum*, planning meeting,
  9-11 December 2003, Miami, Florida.
- 13. Meeting of the Working Group on the ISPM 3 Revision.9-12 December 2003, Rome, Italy.



**TECHNICAL CO-OPERATION PROJECTS** (Highlights of some TCPs)

The Subprogramme has currently technical responsibilities for the following technical co-operation projects that are managed by the Technical Co-operation Department. They fall under five major areas, namely:

- Tsetse
- Fruit flies
- Old and New World Screwworm
- F-1 Sterility for the Control of Lepidopteran Pests
- *Anopheles arabiensis* mosquitoes.

Ongoing Operational Projects are:

ALG/5/019 Control of Date Moth Using the Sterile Insect Technique

BOT/5/002 Support of Tsetse Eradication from Ngamiland

BKF/5/003 Applying Sterile Insect Technique to Create Tsetse Fly Free Zones

EGY/5/025 Area-Wide Fruit Fly Control in Eastern Egypt

ETH/5/012 Integrating SIT for Tsetse Eradication

INT/5/145 Insect Pest Control Using the Sterile Insect Technique

ISR/5/010 Upgrading the Area-Wide Control of the Mediterranean Fruit Fly using the Sterile Insect Technique JAM/5/007 New World Screwworm Eradication

JOR/5/009 Upgrading the Area-Wide Control of the Mediterranean Fruit Fly using the Sterile Insect Technique

KEN/5/022 Integrated Area-Wide Tsetse and Trypanosomosis Management in Lambwe Valley

MAG/5/011 Feasibility Study of SIT-Based Integrated Pest Management of Fruit Flies

MAR/5/009 Control of Diamondback Moth by and Integrated Pest Management System Including the Sterile Insect Technique

MAR/5/015 Feasibility Study For Integrated Use of the Sterile Insect Technique for Area-Wide Tephritid Fruit Fly Control

MEX/5/027 Transfer of Genetic Sexing Mass Rearing Technologies for Fruit Fly Production

MEX/5/028 Prevention Against Cactus Moth in Mexico

MLI/5/017 Integrated Control of Animal Trypanosomosis Through Creation of a Tsetse Fly Free Zone

PAL/5/002 Area-wide Application of SIT for Medfly Control

RAF/5/051 SIT for Tsetse and Trypanosomosis Management in Africa

RAF/5/052 SIT Development for Control of *Anopheles Mosquito* 

RLA/5/045 Preparation for Pilot Fruit Fly-Free Areas using the Sterile Insect Technique in Central America

RLA/0172 An FAO Technical Cooperation Project entitled "Establishment of Mediterranean fruit fly (*Ceratitis capitata*) free areas in Belize, Costa Rica and Panama"

SAF/5/005 Situation Analysis of the Feasibility and Desirability of Tsetse Fly Eradication

In keeping with our policy to highlight activities in a few of our Technical Cooperation projects in each Newsletter the following projects are discussed in this issue: SAF/5/007 Expanding the Use of the Sterile Insect Technique against Fruit Pests in the Western and Northern Cape

THA/5/046 Area-Wide Integrated Control of Fruit Flies

UGA/5/024 Integrated Area-Wide Tsetse Eradication Programme in the Lake Victoria Basin

URT/5/019 Support to National Tsetse and Trypanosomosis Management.

### Prevention Against Cactus Moth in Mexico (MEX/5/028)

The cactus moth. Cactoblastis cactorum (Pyralidae), once the best example of successful classical biological control of weeds (solving a major cactus problem in Australia), invaded Florida in 1989 and has been spreading along the Atlantic and Gulf of Mexico coasts. C. cactorum has become a serious threat to the high diversity of Opuntia (cactus pear) species throughout the world, both native and cultivated. Its presence in the Caribbean and its rapidly expanding range in the southeastern USA, is an imminent threat to the southwestern USA, Mexico, and Central and South America and eventually to other regions of the world. Opuntia cacti are regarded as extremely important plants, especially in arid and semi-arid regions where few plants can be grown. These plants have a subsistence valuable role in and commercial agriculture, in maintaining the ecological balance of unique ecosystems, and in soil conservation and combating Increasing desertification areas with Opuntia cultivation and reliance on food and income from its products, means that invasion by C. cactorum has the potential to impact large regions and thousands of



subsistence farmers in Central and South America, the Mediterranean, North Africa and in other countries. This impact would result in serious social and economic problems.

Impacts on biodiversity and ecosystems where *Opuntia* are dominant components of the vegetation, including the center of *Opuntia* radiation, Mexico, also loom large. The critical nature of this threat, and timing and scale of the likely response needed, requires immediate action. Although the emphasis may initially focus on Mexico, Cuba, other Caribbean islands and the USA, this does not mean that the threat is less important in other countries. Any effective contingency/control programme will need to be approached on a regional or even an interregional scale.

In July 2002, FAO and IAEA hosted a planning and co-ordination meeting (including representatives of environmental organizations) to raise awareness of this major environmental threat and the potential of the Sterile Insect Technique (SIT), and to assess the role  $SIT/F_{1.}$ 

The Joint FAO/IAEA Division has granted a number of research contracts to research institutes in South Africa and the United States who are working in developing the basic technological package for effective survey and control of this pest including a female pheromone for population monitoring and development of artificial diets and radiation biology which are basic elements for future use of the SIT/F<sub>1</sub> sterility.

A relevant result from the radiation biology studies is shown in the Figure 1. Showing the typical response of Lepidoptera sexes to different radiation levels. It is clear that the percentage of eggs from which larvae for the parental generation cactorum of С. were significantly affected by the gender irradiated and by the dose of irradiation. For each gender the percentage of larval eclosion declined significantly as the dose of irradiation increased. This dose effect was greater for irradiated females than for irradiated males. At 200 GY egg hatch was close to zero per cent for irradiated females that mated with normal males, whereas egg hatch was 32% for irradiated males that mated with normal females. These findings confirm the potential to apply  $F_1$ sterility to this pest.



Figure 1. Effect on radiation on *Cactoblastis cactorum* percentage egg hatch (from J. E. Carpenter, S. Bloem and K.A. Bloem. 2001. Florida Entomologist. pp 537-542).

In addition, Mexico presented the IAEA a one-year Reserve Fund Technical Cooperation Project Proposal entitled "**Prevention Against Cactus Moth in Mexico**" which was approved by the IAEA Board of Governors in December 2002 for implementation in 2003. Under this project the following has been achieved:

- A group of five professionals actively involved in the Mexican Cactus Moth Prevention Campaign, travelled to South Africa on a scientific visit to observe cactus moth damage to *Opuntia* commercial crops and to get acquainted with survey and control activities applied in South Africa against this pest.
- A Book Entitled "The Biology, History, Threats, Surveillance and Control of the Cactus Moth, *Cactoblastis cactorum*" was commissioned by IAEA and prepared

by Helmuth Zimmerman, Stephanie Bloem and Hildegard Klein. This book is the first in its kind and is a very significant contribution to the fight against this pest. The book will soon be available in hard copies and on our webpage and will be distributed among interested Member States.

- A 20 minute video in English on cactus moth was commissioned by IAEA as an educational tool and prepared by the of Wit-watersrand. University Johannesburg, South Africa. The video was translated into Spanish by the Mexican Plant Protection Directorate. The video illustrates the main aspect on the biology, damage, survey and control of this pest. The video will contribute in raising the attention of Member States where the pest has recently been introduced and in those that are under the threat of this pest. The video will be reproduced and send to interested Member States.
- A *Cactoblastis cactorum* training course was held in Tallahassee, Florida, from 18 to 20 of November 2003. Participants included: Eight Plant Protection Officials from Mexico and one from the Dominican Republic.
- A cactus moth impact assessment study, commissioned by IAEA, is being prepared by the National Institute of Invasive Species Science, Natural Resource Ecology Laboratory, Colorado State University. This study is needed to show decision makers in public and private organizations in regions/countries at risk, the potential socio-economic and environmental damage that this pest could cause, if no serious efforts are made for containing its spread along its present front of advance along the Gulf of Mexico coast.

Furthermore, the Mexican Government through the Ministry of Agriculture and the Ministry of Environment have been active in preparing the infrastructure to prevent introduction and establishment of the pest in the country and for eradicating an eventual introduction of the pest. A Cactus Campaign has Moth National been recently created by the Mexican Government and prevention activities in high-risk areas, including ports of entry and locations more likely to be affected by natural spread, are underway. A Federal Law allowing for emergency response activities to be enforced in case an outbreak occurs, has been published in the Mexican Federal Register.

In the US, Government Wildlife, Plant Protection and Environmental Agencies and NGOs are aware of the serious consequences of the pest spreading into the Southwest territories and are actively working towards bringing together relevant US organizations to draw plans that will allow facing this invasive species problem in a jointly and coordinated manner.

### Integrating SIT for Creating a Tsetse and Trypanosomosis Free Zone in the Ethiopian Southern Rift Valley (ETH/5/012)

The Ethiopian authorities made progress with advancing the construction of the modular tsetse factory at Kaliti, Addis Ababa. It is anticipated that the construction of the first two rearing modules at Kaliti will be completed early 2004 and that mass production of *G. pallidipes* be started as of April / May 2004. As some difficulties are experienced at Kaliti's temporary insectary with mass-rearing the Arba Minch strain of *Glossina pallidipes*, the Tororo strain of the species, colonized at the FAO/IAEA Laboratory at Seibersdorf, remains the current best option for initiating mass rearing of *G. pallidipes* at Kaliti.

The highly dedicated field teams established very good cooperation with communities in the Southern Rift Valley and in the Deme basin. Doing so, they initiated tsetse and trypanosomosis (T&T) supression in large parts of block one (approx. 10,500 km<sup>2</sup>) of the Southern Tsetse Eradication Project (STEP) area. Although the apparent densities of tsetse in the treated areas appeared to decline as a result of the control efforts, the rapid reestablishment of dense tsetse populations in some areas, where control operations were discontinued for a relatively short time (6–8 weeks), confirms that the approach (use of insecticide impregnated targets and / or pour-on formulations of insecticides) is not reaching the *entire* tsetse population, as this would be required according to the areawide concept of integrated pest management (AW IPM). The technical and environmental feasibility of complementing, prior to the initiation of sterile male releases, the current tsetse suppression activities with other methods, for example sequential (aerial) application of ultra low volume formulations of nonpersistent insecticides (sequential aerosol technique, SAT) needs to be explored.

As the initiation of operational T&T intervention activities and related agricultural and livestock development efforts in the first 10,500 km<sup>2</sup> of the STEP area will necessitate additional funding and support from specialised partners, STEP and the international T&T community, including FAO and IAEA, initiated efforts towards joint international fund raising. In particular a result draft concept note was elaborated and a "road map" for a coordinated international approach of different donors.

### Development of Fruit Fly Pilot Free Zones in Costa Rica to Establish Options for Exports of Fruits and Vegetables Under TCP RLA/5/045.

Costa Rica, as well as the rest of the Central American countries, are producers of a large variety of fruits due to its favourable agroclimatic conditions which, unfortunately, also favour the establishment and spread of pests such as the exotic Mediterranean fruit fly (*Ceratitis capitata*) and other endemic fruit fly species of the *Anastrepha* genus.

The presence of these pests represent a real limiting factor to the production, exports and consumption of fruits, thus effective control is an unavoidable conditions for development of the horticultural industry in countries where these pests occur. The recognition of areas of low prevalence or free of pests, by the International Plant Protection Convention (IPPC) of the Food and Agriculture Organization (FAO), has opened new opportunities for commercialisation of agricultural commodities between countries in the Central American Region and outside the Region.

In 2001, the Central American countries decided to take advantage of the new opportunities for commercialisation of horticultural products and submitted to the International Atomic Energy Agency (IAEA) a Regional Technical Coopration Project (TCP) Proposal for developing fruit fly free and low prevalence areas to establish options for exports of fruits and vegetables. The proposal was approved in that year by the IAEA Board of Governors and an alliance between the Member States and regional and international plant protection organizations was created for implementation and coordination of project activities under the umbrella of the IAEA TCP. The organizations that form part of the alliance are: Interamerican Institute for Co-operation Agriculture (IICA), Food and in Agriculture Organization of the United Nations (FAO) and the United States Department of Agriculture (USDA), through the Moscamed Programme.

Costa Rica, within the framework of the TCP, established in September 2001 a fruit fly survey network in the areas of Hacienda Los Inocentes and sorrounding areas and in Canton la Cruz, Guanacaste, to assess the incidence of Mediterranean fruit fly and some species of the *Anastrepha* genus. In July 2003, the Minister of Agriculture of Costa Rica

officially declared these areas free from Mediterranean fruit fly in accordance with the International Phytosanitary Norms.

The Nicoya Peninsula in Costa Rica, with an extension of 5000  $\text{km}^2$ , presents agroecological conditions which, on one hand, would facilitate eradication of the Mediterranean fruit fly and, on the other, conditions are suitable for commercial production of fruits and vegetables. Given these favourable conditions, in July 2002, the government of Costa Rica decided to initiate an intensive survey to have a clear idea of the fruit fly phytosanitary status in the Peninsula and at the same time to identify locations with potential to be developed into export oriented fruit production areas. A vear later (in August 2003), sterile fruit Mediterranean flies were successfully released in the Peninsula as a pilot demonstration trial for future control activities using the sterile insect technique (SIT).

Credit: This paper appeared in the Official Bulletin "Actualidad Fitosanitaria No. 12" of the States Phytosanitary Service of the Ministry of Agriculture of Costa Rica (M.A.G.) and was prepared by Xenia Carro of M.A.G.

### Mitochondrial Diversity Analysis of *Glossina palpalis gambiensis* from Mali and Senegal Study Partially Funded by Project MALI/5/017

### Extract of IAEA report prepared by:

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*Glossina palpalis gambiensis (*Gpg) is the vector of trypanosomiasis in livestock in Mali and much of the West Africa.

To plan and implement an SIT programme it is necessary to have baseline data on the mobility and tendency of tsetse flies to disperse.

Genetic analysis can be used as indirect measures of dispersal.

Populations of Gpg were sampled in Mali and Senegal in 2002 and

mitochondrial variation was measured in 304 flies from three river drainages in Mali and 69 flies from Senegal.

Results showed that there was substantial gene flow among tsetse from three river systems in Mali, confirming the lack of isolation of the various populations of Gpg. Flies probably disperse widely during the wet season or they may remain in the principle river systems, but following expansion after the dry season they may disperse from linked refugia along seasonal streams with onset of the wet season. Further sampling is necessary to evaluate these possibilities.

### F. REPORTING ON ONGOING AND PLANNED CO-ORDINATED RESEARCH PROJECTS (CRPs) AND RCMs

### Enabling Technologies for the Expansion of SIT for Old and New World Screwworm. 22-26 September 2003, Malaysia. 2<sup>nd</sup> RCM

The 2<sup>nd</sup> RCM of the CRP was held at the Institute Haiwan, Malaysia from 22 to 26 September 2003. The CRP has participants from Iran, Venezuela, USA, Brazil, UK, Indonesia, Sweden and Uruguay. Kluang was chosen because of the presence of an Old World Screwworm (OWS) rearing facility that was constructed in collaboration with the Australian Government as part of its preparedness programme for OWS invasion. The facility was used to produce flies for a small-scale successful field evaluation of the SIT.

The RCM was opened by Dato Dr. Matta bin Abd Rahman, 2<sup>nd</sup> Deputy Director General of Veterinary Services, Malaysia, and Dr. Ahmad Salleh, Director of the Institute Haiwan. The growing importance of the (OWS) was stressed in relation to the planned expansion of livestock rearing in Malaysia as described in a recent economic analysis.

Population genetics of the New World Screwworm (NWS) is an important component of the CRP and many more samples have now been analysed although the sampling is far from complete. Several new laboratory populations from different areas of Venezuela have now been established. The first transgenic strains have now been produced in this species and two microsatellite DNA libraries are at different stages of construction. Progress has also been made on the analysis of sex pheromones and on classical mapping of visible mutations. It was reported that the NWS research activities currently being carried out at the USDA/ARS laboratory in Lincoln, Nebraska will move to Panama early in 2004. It is not clear how this will impact on the implementation of NWS R&D under the CRP.

Population genetics of OWS has been carried out using isozymes, mitochondrial DNA and a nuclear gene, and all the data show a low level of genetic variability over the whole geographic range of this species. These data strongly suggest that OWS is one biological species over the whole of its range, a situation very favourable to any future SIT programme that may be initiated.

Sampling strategy for both NWS and OWS was extensively discussed as it a very important consideration when conclusions from a genetic analysis are drawn. Further population genetic analysis will be essential to enable the geographic source of any new infestations to be identified and a set of sampling guidelines was formulated.

The myiasis fly, *Wohlfahrtia magnifica*, continues to expand its distribution in Crete and Morocco. The infestation in Crete is of recent origin whilst that in Morocco appears to be older and there may have been multiple introductions as there are significant genetic differences between the central and Northern populations.

Fellowship exchanges between laboratories involved in the CRP have been completed since the last RCM and work has started on the development of polytene chromosome analysis for NWS. A website for the CRP is now available <u>http://www.ex.ac.uk/~jrsteve0/iaea/ind</u> <u>ex.html</u> The next meeting of the group will be in early 2005 and it was suggested that this would be in Montevideo, Uruguay.

### Use of Nuclear Techniques in Biological Control. 3-7 November 2003, Vienna, Austria. 3<sup>rd</sup> RCM

Considerable progress was reported in relation to the various applications of nuclear techniques in biological control:

#### A. REARING

1. <u>Artificial Diet Sterilization: Use,</u> <u>Storage and Extension of Shelf Life</u>

**Problem:** Artificial diet often has a limited shelf life and table life.

**Progress:** Artificial diet often has a limited shelf life. Gamma radiation and X-rays can provide a non-destructive means of killing microbial contaminants that may degrade artificial diet or impair insect growth. Initial research has indicated that for situations where prolonged shelf life is not required, freezing or ultra low freezing may be simpler and more efficacious. However, situations during rearing where longer shelf life is required still need to be further explored.

2. <u>Suppression of Host Immune</u> <u>Reactions</u>

**Problem:** Host immune reactions may reduce rearing efficiency or prevent the use of factitious or non-habitual hosts

that are easier or more economical to rear.

**Progress:** Initial data showed that irradiation of Galleria mellonella larvae with doses from 60-80 Gy allowed development of normal the endoparasitoid Venturia canescens while extremely limited development is possible in the non-irradiated larvae. Irradiation of *Plodia interpunctella* and Ephestia kuehniella larvae with doses of 400 and 600 Gy increased emergence of V. canescens compared to non-irradiated controls. This effect might be due to suppression of host immune response in the host larvae. Irradiation of  $\overline{4}^{th}$  instar larvae Chilo infuscatellus with a dose of 60-80 Gy enhanced parasitization by *Cotesia flavipes*, thus achieving a higher productivity of parasitoids in the laboratory.

3. <u>Extension of Storage Time for Hosts</u> or Prey

**Problem:** Normal host/prey development limits storage of host/prey material.

**Progress:** Studies on *Ephestia kuehniella, Spodoptera litura, Sitotroga cerealella*, and *Musca domestica* showed that irradiation caused a prolongation in the development of host stages suitable for parasitization, thus facilitating the use of these hosts under mass rearing conditions. Doses between 75 and 150 Gy used to inhibit potato sprouting can also increase the incubation period of potato tuber moth *P. opercullela*.

### 4. Extension of Host Suitability

**Problem:** Normal host development limits the time interval when a host is suitable for parasitization.

**Progress:** Results have shown that radiation can be used to delay normal insect development and extend the time when a given host stage is suitable for use by parasitoids. These examples are related to the use of parasitoids in poultry production to control Musca domestica, to control Anastrepha spp. in mixed fruit orchards, and to control Ephestia kuehniella and Plodia interpunctella in mills and warehouses. In addition, radiation has been used to extend the time Sitotroga cereallela as factitious host is available for Trichogramma chilonis to control Chilo infuscatellus and two other sugarcane borer species, Tryporhiza nivella and Emmalocerra depressella. Irradiation of Anastrepha spp. 3<sup>rd</sup> instars with a dose of 45 Gy allowed extending the parasitisation period and increase under mass rearing conditions the quantity and quality of D. longicaudata produced. Irradiation of *B. carambolae* eggs with 30-50 Gy extended the larval period available for parasitization of P. incisi. Irradiation of the uzi fly pupae with doses ranging from 0.5-8 Gy allowed extending the parasitization period and increased the progeny production of N. UV-sterilization of the host thymus. eggs markedly increased the parasitization capacity of T. chilonis with respect to unsterilized host eggs of Corcyra cephalonica and S. litura, and

further emergence of the parasitoid from UV-sterilized host was significantly enhanced. Irradiation with 250 Gy to *H. armigera* eggs can prolong the period of parasitization by *T. chilonis*. The developmental time of  $F_1$  larvae of *L. dispar* irradiated in the first instar was extended, thus extending the period available to the natural enemy complex.

### 5. <u>More Efficient Utilization of Sub-</u> Products from SIT Mass-Rearing

**Problem:** Insect mass-rearing programs often produce relatively large numbers of substandard material or have excess production of particular life stages that are discarded.

**Progress:** Discard material may be irradiated and used to support the production of natural enemies. Research has shown that this approach is in fact viable. Examples include the use of excess egg production in *Ceratitis capitata* and *Anastrepha ludens* massrearing facilities to produce egg parasitoids, as well as the use of remnant larvae and pupae to produce larval and pupal parasitoids.

6. <u>Physiological and/or Behavioural</u> <u>Stimulation Effects of Low Doses of</u> <u>Radiation to Natural Enemies</u>

**Problem:** Field performance of laboratory-reared parasitoids and predators is a concern.

**Progress:** In *Trichogramma evanescens* and *Habrobracon hebetor* a low dose of radiation increased adult longevity and oviposition when parasitizing 2-day old *E. kuehniella* eggs irradiated with 20 and 40 Gy for *T. evanescens* and 7.5 to 15 Gy for adults *H. hebetor*. The effects are manifested mainly in the females of *H. hebetor*. In *Trichogramma chilonis* a

low dose of radiation (100-200 mGy) altered the sex ratio in favour of females. Irradiation of adults V. canescens with 3 Gy significantly increased the rate of parasitism of E. kuehniella larvae. When infective juveniles of the nematode entomopathogenic Steinernema glaseri were irradiated and bioassayed one week post-irradiation faster host morbidity and mortality was observed at doses of 1.5 and 2.5 Gy (the effect being distinctly evident at 2.5 Gy). Increased harvesting of irradiated infective juveniles of S. glaseri at 2.5 Gy was also noticed when compared to the control

### 7. <u>Use of Radiation as a Tool to Study</u> <u>Host-Natural Enemy Interactions</u>

**Problem:** Behavioural and physiological interactions between host and parasitoids are complex, often difficult to study and not well understood.

**Progress:** Irradiation of *Glyptapanteles* liparidis wasps (24, 48, and 96 Gy, respectively) caused temporary and sterilization reduction а in oviposition. Irradiation reduced total number of eggs laid per female but did reduce longevity. Effects not of "pseudoparasitization" of Lymantria dispar larvae by irradiated female wasps (i.e. injection of sterilized eggs together with polydnavirus and venom) indicate that this method can be used to study the influence of parasitoid associated factors.

Pseudoparasitism of *L. dispar* larvae caused delayed larval development, morphological abnormities and high mortality during pupation or in the pupal stage. The immune response (hemocytic encapsulation and hemolymph melanization) of the host larva was suppressed by pseudoparasitization. The technique was used in experiments separate parasitoid larvae from to associated factors in vitro and recombine them by implanting them into new hosts. Pseudparasitization prevented encapsulation of implanted parasitoids; but only the complete recombination of larvae with both, teratocytes and polydnavirus/venom allowed successful emergence of parasitoids. Separation and recombination of parasitoid and associated factors is currently used to study parasitoid-induced alterations of juvenile hormone metabolism of L. dispar.

Pseudoparasitization was used to study interaction of *L. dispar* host larvae with various entomopathogenic microsporidia. It slightly but significantly increased spore production of the studied microsporidia in the host. However, semi-permissive hosts did not become fully permissive.

### B. HANDLING-SHIPMENT-RELEASE AND TRADE

1. <u>Reproductive Sterilization of</u> <u>Host/Factitious Hosts/Prey</u>

The Problem: Continued development and emergence of non-parasitized fertile hosts, factitious hosts, as well as of unused prey (pest) insects during rearing of natural enemies often require additional steps in handling, thereby decreasing the efficiency of rearing systems.

**Progress:** During rearing of natural enemies it is often the case that not all of the host material is parasitized or consumed, requiring additional steps in handling prior to shipment of the natural enemies to prevent the release of pest insects. Radiation has been successfully used to reproductively sterilize *Ephestia kuehniella*, *Plodia interpunctella*, Spodoptera litura, Bactrocera oleae the sugarcane borers in order to rear their parasitoids. Radiation has also been used to sterilize the hosts used to rear parasitoids of *Musca domestica*. This technology is also being implemented for the mass production of two larval fruit fly parasitoids, Dichasmimorpha longicaudata and Doryctobracon *crawfordi*, being shipped to a number of Another option for the countries. prevention of the emergence of the host in the production of D. longicaudata could be the irradiation at 25 Gy of eggs of A. ludens.

These uses of natural enemies, without the simultaneous release of pest insects, have only been made possible by the application of radiation. In addition, the application of radiation to the production of natural enemies has reduced handling costs, allowed for earlier shipping, and provided a cleaner product to the customer.

### 2. <u>Provisioning Shipments of Natural</u> Enemies with Sterilized Artificial Diets

**The Problem**: Artificial diets have a short table-life and, as such, are problematic to use for rearing and shipment of natural enemies.

**Progress:** Research has not been carried out in this area. However, it is envisioned that radiation can be used to preserve the quality of the diet over longer time periods, thereby extending the acceptability and suitability of the artificial diet to the natural enemies during shipments.

### 3. <u>Provisioning Shipments of Natural</u> Enemy with Sterilized Host/Prey

**The Problem**: There exists a real or perceived risk that shipping natural

enemies with host/prey material will lead to introduction of non-native, pesticide resistant or new strains of pest insects into new areas or countries. This may exacerbate the ever-stricter quarantine regulations required to obtain permits for natural enemy shipment.

**Progress:** Research on the use of pest mite T. urticae eggs to provision shipments of several species of predatory mites has confirmed that radiation at a dose of 280 Gy or less, depending on the age of the host eggs, can be used to eliminate the risk of introducing fertile pest mites or other insects, or other hitch-hiking arthropods. and at the same time allow the inclusion of nutritional supplements in the form of host material to maintain quality. This application of radiation will help facilitate and encourage the national and international trade of natural enemies.

4. <u>Shipping of Sterilized Pests/Factitious</u> <u>Hosts or Prey (in the Absence of Natural</u> <u>Enemies)</u>

**The Problem**: There exists a real or perceived risk that shipping fertile hosts or prey material will lead to accidental introduction of non-native, pesticide resistant or different strains of pest insects into new areas or countries. This may exacerbate the ever-stricter quarantine regulations required to obtain permits for shipment of insects.

**Progress:** There are commercial needs and opportunities to ship sterile host/prev material in the absence of natural enemies for use and redistribution by smaller rearing facilities and to standardize host material to insure product quality. No research has been carried out in this area, however, it is known that some commercial biological control

companies are making use of this technology. One potential example is the shipment of sterile *Musca domestica* pupae to rear their parasitoids in other locations.

C. FIELD APPLICATION OF REPRODUCTIVELY STERILIZED HOSTS TO ENHANCE BIOLOGICAL CONTROL

1. <u>Combination of Augmentative</u> <u>Releases: SIT/F<sub>1</sub> Sterility + Natural</u> <u>Enemies</u>

**The Problem:** Agricultural and forest production is adversely affected by many insect pests which have traditionally been controlled with a heavy emphasis on chemical pesticides. Resistance to most insecticides has been documented, leading to increased use rates, which in return can exacerbate the adverse affects of pesticides on the environment.

**Progress:** Nuclear techniques (SIT/F<sub>1</sub> sterility) and augmentative releases of natural enemies are compatible strategies that can yield both additive and synergistic effects. Laboratory studies and field trials with Helicoverpa armigera, Helicoverpa zea, Phthorimaea Lvmantria operculella. dispar. Spodoptera litura, Spodoptera exigua, and Plutella xvlostella indicated that progeny from irradiated moths were acceptable as hosts for egg and larval parasitoids. Experimental proof under laboratory conditions showed that F<sub>1</sub> sterility and Trichogramma releases are effective in suppressing P. opercullela. In addition, L. dispar larvae that were reproductively sterilized by irradiation were found to be suitable carriers for the transmission of nuclear polyhedrosis virus to field pests populations. Laboratory and field cage results suggest

that this may also apply for codling moth and false codling moth.

2.	Supp]	lement	Нс	osts	for	Natural
Ene	mies	Prior	to	Pes	t F	opulation
Out	break					*

**The Problem:** Many insect pests have demonstrated cyclic population outbreaks. Although these outbreaks may be predicted, effective and environmentally friendly control strategies are needed to reduces the effects of these economically damaging events.

Progress: Pests with demonstrated cyclic population outbreaks may be controlled if the number of natural enemies could be increased prior to the outbreak. Releasing sterile insects as hosts for the natural enemies could increase the number of natural enemies without increasing the risk that the released pest will cause economic damage in the future. Doses of radiation required to reproductively sterilize pest eggs and larvae were determined. Irradiated *Lymantria dispar* eggs, as well as  $F_1$  eggs and larvae resulting from irradiated parents were studied in the field and found to be acceptable and suitable as host for natural enemies.

3. <u>Supplemental Hosts for Seasonal</u> <u>Maintenance of Natural Enemies</u>

**The Problem:** Pest populations can vary greatly from generation to generation. During periods of low pest densities, population levels of their natural enemies can be reduced to very low levels. The low population levels of natural enemies are then unable to effectively respond in a timely manner to the next outbreak in the pest population. **Progress:** During periods of low pest densities, population levels of natural enemies can be very low and unable to respond to an increase in the pest population. Irradiated Helicoverpa armigera and Plutella xylostella moths released in the field laid eggs that served as host for feral egg parasitoids and caused the parasitoid population to increase. The provision of supplemental hosts to T. chilonis in the field enhanced the survival of the parasitoids in sugarcane fields, which proved effective for the management of several species of sugarcane borers. Eggs laid by false codling moths (FCM) treated with gamma radiation were found to be acceptable and suitable for development of the egg parasitoid Trichogrammatoidea *crvptophlebiae* under laboratory conditions. Field-cage evaluations are planned for early 2004 to ascertain the effect of combined releases of FCM F<sub>1</sub> sterility and Tcrvptophlebiae in South Africa. A pilot study in 25 ha of citrus where both technologies will be applied together is planned for October 2004.

### 4. <u>SIT Against Natural Enemy Pest</u>

The Problem: In certain cases where an insect provides a useful service, natural enemies of the useful insect are considered to be a pest. Examples of would this relationship include parasitoids of the silkworm, Bombyx mori. Varroa mites that attack honeybees, or natural enemies of weed herbivores.

**Progress:** In certain cases where an insect provides a useful service, natural enemies of the useful insect are considered to be a pest (example: parasitoid of *Bombyx mori*, the uzi fly). Radiation biology studies were

conducted to determine the optimum dose required to sterilize the natural enemy pest (uzi fly).

D. FACILITATION OF CLASSICAL BIOLOGICAL CONTROL AND NATURAL ENEMY MONITORING

1. <u>Use of Reproductively Inactivated</u> <u>Agents for Final Confirmation of Host</u> <u>Specificity of Potential Exotic Biological</u> <u>Control Agents</u>

**Problem:** The importation of exotic natural enemies, particularly insect herbivores of plant pests, is becoming increasingly difficult due to concerns over the possibility that imported natural enemies may shift and become pests of beneficial or protected species. In some cases, despite extensive and positive prerelease studies, promising biological control agents are ultimately rejected because of remaining doubts about their host specificity.

**Progress:** A model system including Opuntia spp. and the cactus moth (Cactoblastis cactorum) has been developed to study the host range of an exotic herbivore. Radiation biology studies have been initiated to determine the optimum dose at which females are sterilized and males remain partially fertile and produce sterile progeny. The sterilizing doses of radiation for males and females have been determined. The level of F<sub>1</sub> adult sterility resulting from substerilizing doses applied to the parents are under evaluation. Other potential model systems are being considered including a Pyralidae for the biological control of Brazilian pepper tree currently in quarantine in Florida.

2. <u>Use of Sterilized Hosts for</u> <u>Exploration of New Natural Enemies</u> <u>and for Monitoring Natural Enemy Field</u> <u>Populations</u> **Problem:** The collection of new exotic natural enemies or the monitoring of field populations of native natural enemies is sometimes complicated by the fact that hosts are rare or difficult to locate.

**Progress:** Monitoring of field populations of natural enemies is sometimes complicated by the fact that hosts are rare or difficult to locate. Radiation biology studies were conducted to determine appropriate doses to reproductively inactivate certain Lymantria dispar larvae life stages. resulting from male parents irradiated at 30 and 120 Gy were used in the field for monitoring the number and type of natural enemies (parasitoids and

pathogens). Reproductively inactivated larvae (400 and 600 Gy) of Ephestia kuehniella and Plodia interpunctella were placed in warehouses and mills to monitor the number of *V. canescens* and *H. hebetor* throughout the season. Sterilized larvae of *Anastrepha* spp. and *Bactrocera* spp. will be used to monitor populations of natural enemies. The doses reproductively inactivate to *cactorum* have been Cactoblastis determined. Experiments are planned to determine whether sterile egg sticks (laid by irradiated adults) can be used to survey for generalist egg parasitoids occurring in the generally infested area.

### *New CRP* on "Molecular Technologies to Improve The Effectiveness of SIT" 19-23 January 2004, Vienna, Austria. 1<sup>st</sup> RCM

The past ten years have seen an explosion in the use of molecular biology in all biological sciences; especially in the fields of medicine and agriculture and particular emphasis has been placed on gene transfer technology. The recognition that the development of gene transfer techniques in pest insects may lead to improvements in the SIT, encouraged the Joint FAO/IAEA Division to support and co-ordinate activities in this field by funding two

CRPs. Scientific progress in the field is now such that transgenic technology in pest insects can be moved from the laboratory to initial evaluations of strains under operational conditions of large scale rearing and in contained field-cage situations to assess mating competitiveness of the transgenic strains. The new CRP will address these areas with the objective of delivering improved transgenic strains for eventual use in SIT programmes.

The two previous CRPs have provided the groundwork for this new proposal that will facilitate moving the field of the use of transgenic insects from the laboratory towards field application. During the implementation of the previous CRP highly efficient vectors for pest insect transformation have been developed and widely applicable transformation markers have been identified. These developments have made screening of putative the transgenic individuals in any pest species extremely efficient and have led to the creation of transgenic strains in more than 10 pest insect species. In many pest insects molecular analyses of important gene systems have produced useful biological reagents for the development of transgenic strains. The project will involve transfer of transgenic strains to larger rearing situations, evaluation in contained fieldcage conditions and initial model studies on the impact of transgenic insects on the environment.

The new CRP will also begin the first major studies on the biological fitness of transgenic insects. Whilst not directly focused on risk assessment issues, the data produced will be of considerable importance to the development of a regulatory framework for any eventual open field release of transgenic insects. In order to maximize the chance that transgenic technology can be effectively transferred field to programmes, participation of operational SIT managers, regulators, ecologists and other stakeholders will be encouraged.

Overall objectives of the CRP:

To move transgenic insect technology from the laboratory gradually towards the field for the eventual management of insect pests through the improvement of the SIT.

Specific objectives:

- To improve gene transfer technology for the safe and effective use of transgenic or paratransgenic insects in pest management.
- To assess the performance of transgenic strains in large-scale rearing and under contained field-cage conditions.
- To define ecological and population concepts and models associated with the release of

transgenic insects for pest management.

Expected research outputs:

- A collection of characterized molecular reagents and promoters/regulatory elements to be used for the expression of effector genes or selectable markers in pest insects.
- Increased knowledge of molecular mechanisms of sex determination and dosage compensation in pest insects.
- Data on the interaction between the transgene and the whole host genome in target pest insects.
- Development and definition of systems to maintain stability and strain integrity under large scale rearing.
- Transgenic strains of pest insects including: a) genetically marked strains, b) male producing strains and c) refractory strains.
- Data on transgene stability and fitness of transgenic strains during large scale rearing and contained evaluation.
- Mathematical models to predict the interactions between transgenic and wild type populations in the environment.
- Knowledge and data to support risk assessment analysis for the eventual release of transgenic insect strains.

Expected duration: 5 years (2003-2008); first RCM is planned **19-23 January 2004, Vienna, Austria**.

Details of the IAEA Research Coordination Programme can be found in the IAEA web site: http://crpdev.iaea.org.

### *New CRP* on "Improving Sterile Male Performance in Fruit Fly SIT programmes" 25-29 October 2004, Guatemala City, Guatemala. 1<sup>st</sup> RCM

Following a Consultants Meeting recommendation (see Section H), the IAEA Research Contracts Committee has approved the initiation of a new CRP in 2004.

The objective of the proposed CRP is to ultimately reduce the cost and increase the effectiveness of SIT programmes by improving the performance of sterile through manipulations males implemented at the emergence and release facilities. Thus, the focus of the proposed CRP will be the developmental period from the late pupal stage of massproduced fruit flies through to field release. The proposed CRP will focus on the ten tephritid fruit fly species currently the target of operational SIT programmes, and thus is very distinct from another CRP foreseen to start in 2005 involving the development of antecedent rearing process control procedures for fruit fly pest species for which the SIT is not yet available.

### a) The Abiotic Environment

From the time that the sterile fly pupae reach the distribution centres, they are subject to the abiotic environment into which they are placed. This environment includes the type and size of the fly holding container, and the specific ambient conditions of the holding room. The type of holding container will depend on the kind of fly

release that will follow-either from the ground, or by air. Containers for ground release are typically small, either bags or boxes, and hold up to several thousand individuals. Fly density and the amount of surface area available for flies to rest on are important factors to consider in order to avoid overcrowding and stressing the flies. An advantage of ground-released flies is that once the fly pupae are set up they require no further handling. except perhaps to add additional food or water. Aerial release of flies is usually more complicated, as the flies must be transferred from initial holding containers to aerial drop boxes by temporarily anaesthetizing the flies, usually with cold. An exception to this procedure is the use of small bags or boxes, which can be loaded directly into plane and torn open at the time of release. The latter may be needed, or alternative methods of anaesthetizing the flies, in cases where a cold fly knockdown procedure is a problem, as recently described for 2 species of Anastrepha (ludens and suspensa) (P. Teal, unpub. data), and for Bactrocera papayae (Tan & Nishida, 1998) and B. carambolae (Wee & Tan, 2000). In these cases, male pheromone quantity or quality is affected by even brief cold treatments to young adults.

The ambient environmental conditions in the fly holding room are also potentially critical. Such parameters as light intensity, the light:dark cycle (if any), temperature, humidity, and barometric pressure may all play important roles in conditioning the flies for their vital role in the field: surviving and attracting, courting, and copulating with wild females. Much remains unknown about the effects of manipulating these environmental parameters, either separately or in combination, on subsequent male quality in the field.

### **b)** The Biotic Environment

### b.1. Hormones

Age is a significant factor affecting sexual signalling and reproduction in numerous tephritid species. For example, members of the Anastrepha genus typically require between two and three weeks to become sexually mature. Although mass rearing results in selection of strains which become sexually mature much earlier than wild flies, the most rapidly developing strains of A. suspensa and A. ludens still require more than 7 days to become sexually mature This delay between adult emergence and sexual maturity poses a significant problem for SIT programmes because males must be held for a long period of time prior to release, or have to be released before becoming sexually mature, resulting in fewer surviving to maturity and copulation.

Clearly, development of cost effective methods to accelerate sexual maturity in released flies would have a significant positive impact on the efficacy of the SIT. Recent research on a number of tropical *Anastrepha* species (*A. ludens*, *A. suspensa* and *A. obliqua*) has shown that juvenile hormone is a critical hormone regulating sexual maturity and sexual signalling in these species (e.g.

Teal et al., 2000). Topical application of juvenile hormone or the mimics methoprene or fenoxycarb, accelerates reproductive development and sexual signalling in sterile males by at least 5 days. Incorporation of methoprene into adult diets results in similar acceleration of development. Furthermore, sterile males fed methoprene release significantly more pheromone and are considerably more attractive to fertile females between the ages of 3-11 days than are sterile males denied access to hormone in the diet. Additionally, significantly more wild females are attracted to sterile treated males (6-10 days old) fed hormone than to wild males (16-20 days old) fed the same diet, except lacking hormone on each day of a four-day test. Effects of methoprene are optimal when as little as 0.05% (AI) is incorporated in the adult diet. This. coupled with the relatively low cost associated with the purchase of methoprene in water-soluble а formulation, indicates that incorporation of hormone supplements into adult emergence protocols may be a cost effective way to improve the efficacy of the SIT.

### b.2. Nutrients

Both male and female tephritids are anautogenous, emerge as adults with undeveloped gonads, and relying on foraging during adult life to provide the protein needed for gonadal and accessory gland development (Yuval & Drew, 2000). In addition to protein, carbohydrates must be frequently ingested to fuel metabolic activities. Recent studies on species from several tephritid genera (Anastrepha, Bactrocera, Rhagoletis and Ceratitis) indicate that providing protein nutrition to males in the days following eclosion can enhance male reproductive success. These studies have been extended to sterile male Mediterranean fruit flies, establishing the *Ceratitis capitata*, potential for including protein in the diet offered sterile males in the release facility (Kaspi & Yuval, 2000), although the optimal dosage and form of presentation still needs to be established (Papadopoulos et al., 1998; Shelly & Kennely, 2002). Furthermore, recent studies indicate that several species of bacteria are common residents in the tephritid gut, and may make a significant contribution to fly fitness (Drew & Yuval, 2000; Lauzon et al., 2000).

Currently, sterile males of most species are usually offered a pre-release diet of highly concentrated sucrose, presented in an agar block. The formulation and testing of optimal pre-release diets, containing sugar, protein and bacteria (and possibly other ingredients) in proportions that will result in enhanced sterile male performance in the field, will be developed in the proposed CRP.

### b.3. Semiochemicals

Most pest species of *Bactrocera* are attracted to two major natural attractants, raspberry ketone (RK) and methyl eugenol (ME) (Tan, 2000 a & b; Tan et al., 2002). Species attracted to RK, such as *B. cucurbitae* and *B. tryoni*, sequester the chemical into the pheromonal system. However, the process of sequestration of RK has, thus far, not been observed to contribute significantly to the improvement of male mating or competitiveness in courting females.

Ingestion of ME by *Bactrocera dorsalis* has been studied intensively. ME is converted to two major booster sex

pheromonal components - trans coniferyl alcohol and 2-allyl-4,5-dimethoxphenol in the crop (Tan, 2000). The metabolites are stored in the rectal gland and subsequently released during fanning performed during courtship. These improved components the mating competitiveness of males by at least three fold when compared with MEdeprived males. It is envisaged that providing sterile males with a source of ME to feed on before release will place them on at least an even playing field against wild males, thereby potentially reducing the number or frequency of sterile males released. To date, the role of ME as a precursor to boost sex pheromone in *B. correcta* and *B.* philippinensis is not fully understood nor whether improving male production mating competitiveness can be realized.

Other advantages of ME feeding are related to survival. One of the components, 2-allyl-4,5-dimethoxyphenol, acts as a very potent allomone to deter vertebrate predators (Tan & Nishida, 1996; Wee & Tan, 2001). Furthermore, feeding ME on significantly reduces male response to ME in male annihilation traps, thus potentially allowing simultaneous application of the SIT and male annihilation methods.

Recent findings have shown that ginger root oil and the oil contained in the flavedo region (peel) of citrus fruit enhance considerably the mating competitiveness of wild or mass-reared *C. capitata* males (Katsoyannos et al., 1997; Papadopoulos et al., 2001; Shelly, 2002). Once exposed (by contact or vapour), males acquire a strong mating advantage over unexposed males and retain it for a number of days. Interestingly, commercial essential oils from citrus have a similar effect on males.

These oils are especially and at the same time very effective, so it would be worthwhile trying their potential in ongoing SIT programmes. If sterile become males more competitive following exposure to the oils a smaller number of them would be required to be released. One active ingredient in ginger root oil has been identified as  $\alpha$ -copaene, a potent male attractant. However, these oils contain a number of other volatile substances that may have similar enhancing effects on males. The composition of citrus peel oil and the effects of the various components must be examined.

There are no known effective mating enhancing semiochemicals for *Anastrepha*. However, the dramatic mating enhancing effects seen with *Bactrocera* species and *Ceratitis* suggest that similar compounds may exist in the *Anastrepha* ecological sphere and await discovery.

### c. Release Methods

Tephritid fruit flies can be distributed in the field by either aerial or ground release methods. Ground release methods are simpler but much less effective when large or topographically difficult areas need to be covered with sterile flies. Ground distribution can be accomplished by either point or roving releases of flies from containers designed to be handled easily by personnel. In the case of ground releases, flies are released from the same containers into which the pupae are packaged when they are received at the distribution facilities. Commonly, this means the use of buckets each containing several hundred up to a thousand individuals held in environmentally controlled rooms. Larger containers (e.g. PARC boxes) containing several tens of thousands of flies may also be used to make larger point releases in fly 'hot-spot' areas.

Aerial fly releases are necessarily used in large scale SIT programmes around the world. This method invariably means that the pupae arriving at distribution facilities will be held in temporary containers, such as PARC boxes or the more recently developed emergence towers, until just prior to aerial release. In the case of aerial releases, the flies must be extracted from the temporary containers through the use of cold fly knockdown methods, and then transferred to aerial drop boxes, which are transported to waiting aircraft. These methods, while operationally convenient, may not be always optimal in terms of the success of the SIT. For example, as noted above, in some species, (notably Anastrepha ludens, A. suspensa and several Bactrocera species), cold knockdown treatment adversely affects sexual competitiveness. Therefore, one of the goals of the proposed CRP is to integrate the release methods with the other manipulations discussed above, tailoring the release method according to the biology of each fly species.

### Specific R&D Activities

R&D activities will focus on the ten tephritid species currently targeted by active SIT programmes in the different regions of the world. Consequently, basic research needs to be conducted on those species for which the results of such pre-release manipulations are unknown or limited. Concurrently, for species where promising those applications have surfaced, these should be applied, initially in pilot tests and eventually incorporated in standard procedures in ongoing SIT operations. In all these activities, it is of cardinal importance to use the global standards, as established in the international manual on fruit fly quality control (FAO/IAEA/USDA 2003), regarding male performance for the following traits: Survival. Dispersal and Copulation/Insemination.

### 1. Abiotic and Fly Release R&D

The following activities should be undertaken to manipulate the fly holding conditions at the distribution centres, as well as the procedures for finally releasing the flies into the field. Strong emphasis should be placed on resulting fly quality rather than simply operational convenience.

### 1.a Fly holding conditions:

Several ambient environmental parameters, including temperature, relative humidity. barometric pressure and illumination should be varied in controlled experiments and the final field quality of the resulting sterile males The size (volume) of the assessed. containers and resulting fly density should be varied and tested as well. Experiments to pre-adapt late-stage pupae or adults to specific field conditions, e.g. high or low temperature, light intensity, barometric pressure, should be carried out and compared to results from insects held under standard conditions. Of course, the addition of a supplemental treatment such as a hormone or semiochemical (see below) may affect the choice of the most efficient method by interacting with the fly holding conditions (e.g. the size of the container or the manner in which the supplement is applied to the flies).

### 1.b Fly release conditions:

If flies need to be transferred from holding containers to aerial drop boxes, standard and new ways to anaesthetize the flies should be investigated (e.g. use of carbon dioxide or nitrogen gas instead of cold knockdown) for species known to be sensitive to cold treatments. For these species, alternatives to the use of anaesthesia in the first place should be investigated, i.e. transferring the flies directly to awaiting release aircraft, as is the case for ground releases. Since aerial releases are the principal means of sterile fly release in all large-scale action programmes, efforts should be directed to find efficient means of aerially dropping flies for ongoing programmes in which only ground releases are currently operational.

After consuming methyl eugenol, Bactrocera fruit flies require 2-6 hours to convert methyl eugenol to its metabolite(s) and to transport them via the haemolymph to the rectal gland for storage and subsequent release during courtship. During this period, shifting the flies and transporting them for aerial affect the release mav eventual sequestration of the metabolite - thus negating the mating competitive advantage of exposed released flies. As such, research should be conducted to: i) determine whether the process of booster sex pheromonal components reaching the rectal gland can be affected by

chilling; and ii) determine the ideal length of time after post-treatment to chill (if any) and then release sterile flies (this may reduce unnecessary fly retention time in a mass rearing facility).

### 2. Hormonal R&D

Experiments on Anastrepha suspensa and A. ludens have shown the utility of incorporation of methoprene into the adult diet. Sterile males, between 3-11 days old, fed a methoprene containing released significantly diet. more pheromone and were significantly more attractive to fertile females than were sterile males denied hormone in the diet. The juvenile hormone and its mimics have some beneficial effects, but they have been known to induce morphological intermediates-larvalpupal and pupal-adult forms, when larvae and pupae are exposed at critical periods (just before and after pupation). As such, incorporating juvenile hormone or its analogue into larval diet is not feasible. To overcome this problem, one or two day old pupae can be treated with different residual doses of a juvenile hormone or its analogue. If the cost of juvenile hormone is high, the efficacy of some of its commercial analogues should first be analysed. Besides this, the cost effectiveness of residual treatment with the leaves of *Cyperus spp.*—weeds which contain substantial amounts of juvenile hormone III (Toong et al., 1988), an ideal substitute for juvenile hormone, should be investigated. Should residual treatment of pupae with juvenile hormone appear not to be a viable option, incorporating juvenile hormone or its analogue into adult diet may be a viable option to induce early maturity in male flies.

This, coupled with the relative low cost associated the purchase with of methoprene. in а water soluble formulation, indicate that incorporation of hormone supplements into adult holding protocols may be a cost effective way to improve efficacy of the SIT. At this juncture, information available for A. suspensa and A. ludens is sufficient to allow for the :

- Development of efficient large-scale methods for incorporation of methoprene into adult diets.
- Conducting of pilot scale tests that allow assessment of efficacy of the methods.
- Conducting of cost benefit analyses to determine feasibility of the method.

For other target species (*A. obliqua, A. fraterculus, C. capitata* and *Bactrocera* spp.) a significant amount of basic research must be conducted prior to the implementation of hormone supplements into SIT release programmes. These studies should include:

- Behavioural and chemical assessments of the effects of methoprene to determine the age at which males become sexually mature and if the reproductive parameters are sufficiently improved to make the sterile males more competitive with wild flies.
- Determine the minimum dose and length of time required to optimise reproductive performance and minimize time for maturation.
- Assess the efficacy of the technology by comparing mating success of

sterile males with wild females with the former either provided with hormone supplement or not.

- Determine if hormone supplements should be incorporated into adult diets or provided in other ways (spray applications to pupae, provided in water provided to adults, etc.).
- Develop efficient large-scale methods for incorporation of methoprene into adult diets.
- Conduct pilot scale tests that allow assessment of the method.
- Conduct cost benefit analyses to determine feasibility of the method.

### 3. Nutritional R&D

To date several experiments and observations have pointed to the beneficial effect of providing sterile males with protein before their release. The objective of the research proposed for this CRP will be to establish the best source of this protein nutrition and the form in which it is presented to the sterile males. In light of the complex interactions between diet, sexual performance and survival, these studies will strive to find the diet that provides the best cost effective balance between these three factors. In addition, the possibility of using inexpensive, locally available sources of nutrients (such as leftovers from fruit processing industries), to replace expensive yeast hydrolysates will be examined. As work with the medfly progresses towards implementation in field programmes, basic research on post-teneral diets and sexual performance of males in other species will be conducted.

Tephritid fruit flies maintain intimate associations with a complex microflora

resident in their digestive system. While this interaction is largely unknown, it become evident that several has dominant species (such as Klebsiella oxytoca and Enterobacter agglomerans) in this array of microorganisms may contribute significantly to fly health and reproductive performance. Due to the conditions in mass rearing facilities, these important bacteria are not present (at least not in large quantities) in the gut of sterile males at the time of release. One research component of this CRP will be to investigate the utility of inoculating sterile males with bacteria, by quantifying the manner in which such inoculation affects subsequent survival and copulatory success.

#### 4. Semiochemical R&D

### 4.a. Volatile oils

Orange peel oil and ginger root oil exposure to males was found to enhance considerably *C. capitata* male mating ability and competitiveness compared to unexposed males. These findings may have an important impact on the success of medfly SIT programmes and merits further research.

Future research should address:

- The behavioural, physiological and ecological basis of the observed phenomenon.
- The active compounds involved.
- The parameters involved when applying such substances.
- Ways of incorporating them into the pre-release treatments in SIT programmes.
- Possible other effects on fly behaviour including longevity, dispersal and general performance.

- The cost-benefit of incorporating these supplements into SIT operations.
- Other plant derived chemicals with similar effects.

### 4.b Methyl eugenol

Methyl eugenol (ME) is not soluble in water. Since methyl eugenol is beneficial by increasing male sexual competitiveness and at the same time toxic at high concentrations, it is important to find an effective dose of methyl eugenol that simultaneously does not kill male flies and yet is sufficiently high to significantly reduce the tendency of flies to respond to ME sources (e.g. ME monitoring traps). To simulate a plant source of methyl eugenol, a suitable emulsifier (which does not induce mortality) needs to be identified. Emulsions of methyl eugenol of various concentrations can then be tested on male flies to determine the optimum concentration of methyl eugenol to achieve the desired mating competitiveness against wild males.

At sexual maturity, male flies are strongly attracted to and compulsively

feed on methyl eugenol. The peak period of attraction of wild flies to methyl eugenol traps is between 0800 and 1100 hour for *B. papayae* (=*B. dorsalis*) and the feeding period lasts for 45 seconds to 7 minutes. These optimal ranges for feeding periods need to be determined for each target species that may differ from *B. papayae*.

A suitable and cost effective absorbent for dispensing methyl eugenol emulsion needs to be identified through research. With the right absorbent, a proper method of dispensing the methvl eugenol into fly cages has to be order developed in to avoid contaminating male fly bodies (which may lead to death). The dispensing technology employed should be for a short period of less than an hour.

Expected duration: 5 years (2004-2009); First RCM is planned **25-29 October 2004, Guatemala City, Guatemala.** 

The deadline to submit research proposals is *April 2004*; forms for research contracts or agreements can be found under http://crp-dev.iaea.org.



THE **DEVELOPMENTS** AT ENTOMOLOGY UNIT

Tsetse R&D

G.

#### Survival of Glossina pallidipes irradiated and exposed to low temperature

Investigations into the possibility of using the chilled adult release system are continuing as an alternative to releasing sterile tsetse flies in cardboard boxes (Newsletters 58 & 61). Exposure of adult male tsetse flies to low temperature should be for as little duration as is possible so that the fitness of the released sterile flies is not unduly compromised. It is also necessary to ensure that losses are minimised during bulk irradiation of chilled adult flies. After the previous experiments to determine responses of tsetse flies to individual treatments of sterile males, an attempt was made to simulate the real situation of adult male handling in an operational programme. Adult Glossina pallidipes males were chilled prior to irradiation in a cobalt-60 gamma source. For irradiation the flies were placed in small rearing cages or in an insulated polystyrene (Styropore) expanded cylindrical container or thermos flask. The latter two containers fitted snugly into the radiation chamber. Flies were irradiated with a dose of 120 Gy at approximately 20Gy/minute at room temperature and then placed in an incubation chamber for up to six hours at 4.5°C and relative humidity fluctuating about 50%. The flies were then returned to normal colony holding conditions and the flies irradiated in the large containers

apportioned into holding cages to observe survival without feeding. Survival was recorded on the morning after return to normal colony conditions.

Chilling using a thermos flask had a small impact on survival that was nearly similar to chilling in small cages, whereas chilling in the expanded polystyrene container resulted in low survival rates by the following morning after removal from the chilling chamber. It was possible to maintain the flies in an inactive state during radiation when chilled in a thermos flask whereas the flies became active in the expanded polystyrene container by the time irradiation was completed. It was thus determined that it was necessary to use a container that allowed the low temperature environment to persist through the brief irradiation period and brief exposure to higher temperature during transportation. Generally, the survivors were active up to seven days after removal from the chilling chamber without any feeding. This series of experiments has confirmed that a release strategy can be developed which includes the chilling of sterile males for up to 6 hours. The next phase is to include the prototype chilled adult machine in the evaluation.

#### Salivary gland hypertrophy virus

Following the successful viral purification from salivary glands of infected G. pallidipes flies, partial cloning and sequencing of the genome of the salivary gland hypertrophy virus was performed at the (SGHV) Laboratoire de Pathologie Comparee (Prof. M. Bergoin), Univ. Montpellier II, France. Using primers derived from the virus-specific sequences, a PCR-based diagnostic was developed. Dr. H. Bossin, who joined the Entomology Unit last June, has focused his efforts on the use of this very sensitive detection method to detect the presence of SGHV in infected flies in a non-invasive manner. After optimization of the PCR procedure using extracted tsetse DNA that required the fly to be sacrificed, investigations of alternate sources of viral templates leaving the fly intact were initiated. Tsetse meconium (first excretion from the emerging teneral fly), saliva, and pupal cases have been collected and are currently being tested. Although, tsetse saliva has already proven to be a reliable template source for the detection of virus infected flies, its collection is cumbersome and usually affects the fly viability. In its current developing stage the tsetse virus PCR diagnostic tool is already used to test at an experimental scale to see if it is possible to develop a virus-free colony of this species. In addition to these investigations. experiments are underway to understand the specifics of viral transmission under mass-rearing conditions, especially the role played by horizontal transmission through membrane feeding.

### Pupal sexing

The initial attempts to sex pupae, reported in the last Newsletter, proved unsuccessful on further investigation. The apparent division of the spectra into two categories did not correlate with sex, and subsequent spectra did not show the same division. A new collaboration has been initiated with Dr Floyd Dowell, USDA-ARS, through Dr W. Wirtz, CDC, Atlanta. Dr. Dowell's unit in Kansas is involved with the processing and sorting of grain, and they have developed a system utilizing nearinfrared (NIR) spectroscopy to identify corn grains infested with beetle larvae. The system is currently a prototype, but it is due to be commercialized early in 2004. Three version of the machine currently exist, one requiring hand loading and two with automatic feed. Of the automatic feed versions, one handles about 60 samples per minute, the second about 60,000 per minute but with reduced resolution.

This system shows great potential for identifying other insects also, and it was proposed to try the system on tsetse pupae. Three batches of 100 pupae were shipped to Atlanta and forwarded to Kansas. The batches were about 10, 20 and 30 days post larviposition on arrival in Kansas, and therefore about 21, 11 and 1 day before emergence. Using the slower automatic machine all the pupae were scanned and the first batch returned to Atlanta for emergence and sexing. The sex of the first 90 emerged flies was reported back to Dr Dowell to use for calibrating the identification system. Based on this initial calibration, the remaining 7 emerged flies of the first batch of 100 (three were unemerged) were correctly classified. The remaining batches were rescanned at approximately 10 day intervals and returned to Atlanta for sexing. Using the same calibration the second and third batch's sex was predicted with 85 - 90% accuracy, for both the spectra taken just before

emergence and the second batch spectra taken 11 days before emergence.

This work will be followed up with a visit by Dr Dowell to Seibersdorf, which will allow us to take many more spectra and improve the calibration. The ultimate objective to provide a system to sex the pupae at least 5 days prior to emergence, to allow separate handling of males and females, and accurate sex ratio loading of production cages.

### **DNA microsatellites**

extremely useful DNA These are population sequences for genetic analysis and will be valuable tools for developing a good understanding of gene flow in field populations of tsetse. High molecular weight DNA was extracted from G. pallidipes, G. fuscipes, G. tachinoides and G. palpalis and a company was contracted to construct micro-satellite enriched DNA libraries. These have now been produced and a minimum of 25 microsatellite sequences/species has been identified. These sequences can be made available to interested parties.

#### Prototype tsetse rearing container

After extensive testing and adjustment, the temperature and humidity conditions inside the shipping container are now satisfactory for tsetse rearing. A small sample of flies held for three days in the container showed no evidence of any toxic substance or contamination. A small colony of Glossina fuscipes *fuscipes* is now being held in the rearing container to assess overall performance. This will be maintained colony Fruit Fly R&D.

Reproductive compatibility among *Anastrepha fraterculus* populations

independently of the main colony to measure all performance parameters.

### **TPU3-2 drawings available**

The full design details for the latest modification to the Tsetse Production Unit 3 (TPU3-2) are now available on our web site:

(http://www.iaea.org.programmes/d4/ind ex.html) under News on the left navigation bar. The full technical drawings and material specifications are available in Adobe Acrobat® Portable Document Format<sup>®</sup> so that they may be easily viewed and printed without special software. These drawings and specifications are copyright of the Agency, but they may be copied, distributed and utilized so long as the copyright notice is not removed or obscured and no charge is made for such distribution or use. No changes should be made without approval from the Agency. These latest changes involve only changes in the construction detail intended to reduce the cost, and have no effect on the operation of the system.

### A new tsetse rearing laboratory

A tsetse rearing laboratory has been established at the Institute of Zoology, Slovak Academy of Science (IZ-SAS), Bratislava. The Unit provided technical input to the design of the laboratory and is now supplying regular shipments of *G. pallidipes* pupae in order to establish a colony of this important species. The group at IZ-SAS will carry out R and D on blood diet and supply facilities in Africa with tsetse pupae for colony development.

There were clear mating incompatibilities between *A fraterculus* populations sampled from six 6 locations
in Central and South America. Flies Brazilian populations from two (Piracicaba and Bahia), were sexually active at different times of the day and no matings involving the two populations were observed. However, subsequent taxonomy studies indicated that the population from Bahia belonged to the species, Anastrepha sororcula. It was also found that the A. fraterculus from Colombia were sexually active at sunset; the first reported case in which this species was found to mate in the late afternoon. The peak of mating activity of A. fraterculus from Peru was during the midday with the other populations showing a peak in the beginning of the day. However, even within the populations that mated in the morning the degree of sexual isolation could be quite high. The indices of relative performance showed, in general, that the different populations were equally active during the tests. This result strongly supports the suggestion that incompatibility among the populations is not due to a side effect of one population being more sexually active than the other under the test conditions. In addition, taxonomic. DNA and pheromone analyses are being carried out on the different populations. In practical terms, these results strongly suggest that any SIT approach against A fraterculus will have to pay particular attention to the geographic origin of colonies used for sterilization and release.

### Support for SIT technology in Brazil

The Government of Brazil is considering the implementation of SIT technology for fruit flies in the Rio San Francisco Valley, one of the biggest tropical fruit exportation areas in Brazil. A fruit fly mass rearing complex is planned to produce  $250 \times 10^6$  sterile medfly male pupae per week and two fellows from Brazil have been trained in the mass rearing of GSS. During their training, experiments were conducted to improve the thermal treatment for male-only production, and a comparison was made of the OC profile of all available GSS. A first assessment of mating compatibility in field cages between wild flies from Central-North area of Brazil and the Vienna-8 Mix-2003 GSS was carried out. The results of the field cage tests have shown high sexual activity during the test but low level of compatibility between the two strains. These tests will be repeated at Seibersdorf and in Brazil.

In addition, work is continuing to develop diets for *A. fraterculus* and *C. capitata* that are based on ingredients available in Brazil. These ingredients have been shipped to Seibersdorf for testing. Many different larval diets for the two species have been tested on a small scale and a few have been chosen for mass rearing evaluation. Adult diet for *A. fraterculus* is important for egg viability and various products from Brazil are undergoing testing.

# Replacement of genetic background in a medfly GSS

All medfly genetic sexing strains (GSS) were generated with a wild type strain originating from Egypt. This strain was colonised in 1963 and there is concern that this long colonization period may prove detrimental to the GSS. Generally, three arguments are considered why the genetic background of the GSS needs to be exchanged: a) mating incompatibility with target populations other than Egypt, b) host preference and c) genetic diversity. However, it was demonstrated that with the potential exception described below none of the wild type populations tested so far showed any mating incompatibility with GSS. With respect to the second argument, it is believed that host preference is determined more by the seasonal availability of hosts rather than by genetic factors. The third argument may be relevant as genetic diversity is considered to be an important factor for the overall vigour of the insects. Although direct experimental no evidence exists that shows the benefit of outcrossing on, for example, mating performance, the sexing strains are generally outcrossed with a mixture of different wild type populations before being supplied to facilities.

One potential case exists where a GSS seems to show some incompatibility with the target population. Field cage tests with wild flies from Madeira island showed that GSS males mate less efficiently with wild females than it would be expected from the data that were obtained in the many tests with other wild populations. To try to solve this problem, crosses were initiated to replace the genetic background of a GSS with genetic material from Madeira. This required that the two strains that carry the two components of a GSS, i.e. the homozygous wp tsl strain and the translocation strain, have to be backcrossed repeatedly with a wild type strain from Madeira. Each consecutive cross reduces the amount of remaining Egypt material by 50%. However, this applies only for the chromosomes that are not involved in the sexing translocated mechanism. The Y chromosome will not be exchanged at all and the two 5<sup>th</sup> chromosomes, one attached to the Y and the other carrying wp and tsl, will be replaced only partially if recombination with а Madeira homologue occurs. However,

recombination between the wp tsl chromosome and its Madeira counterpart can also lead to the unlinking of these two markers, i.e. the tsl mutation can be lost. To avoid this a crossing scheme 5<sup>th</sup> was devised where first а chromosome balancer (labelled with the homozygous lethal mutant, Sergeant,  $Sr^{2}$ ) is outcrossed with Madeira material. Such a balancer chromosome carries multiple inversions covering virtually the entire length of chromosome 5 and thereby renders most or even all recombinants inviable.

Currently this cross is in generation 11 and it can be calculated that only 0.05% of the original Egypt background is still present. In the next phase the outcrossed balancer strain will be crossed with the translocation strain that was outcrossed in parallel to the Madeira strain. This produces translocation males cross where the free autosome is labelled with  $[T(Y;5)/balancer-Sr^2;$  $Sr^2$ Madeira background] and these will be used to outcross the homozygous wp tsl strain (D53). In this way the wp tsl chromosome is always opposite to the balancer and, secondly, the flies that carry the wp tsl can always be detected and used for the following cross. Such a scheme is necessary because each outcrossing step dilutes not only the Egypt background but also the number of *wp tsl* chromosomes in the outcrossed population. If the scheme is used as described here, the resulting strain is labelled with also а specific mitochondrial haplotype that can be used to differentiate this strain from most wild populations, e.g. this internal marker can be used to detect contamination of the mass reared colony or it allows to determine whether insects detected in the field are from the target population

or are released flies. The construction of

#### Mosquito R&D

The mosquito rearing facility was completed in June and a colony of *Anopheles arabiensis* has now been successfully established. Initial studies are being carried out on the basic rearing parameters for this species and the first fellow has completed his studies on water quality assessment and its impact on larval rearing. A colony of *An arabiensis* from Reunion has been established in Durban, South Africa for subsequent transfer to Seibersdorf. A new staff member, Mr B. Knols, from the Netherlands joined the mosquito group in July and as of 2004 the mosquito activities will be funded from a new Agency project. This will enable a small expansion of the group to take place. It is planned to hold a consultant's meeting on mosquito rearing in the first quarter of 2004.



### **Consultants Meetings**

# Consultants Meeting on "Development Of Guidelines for Verification of Tsetse Fly Free Areas and the Trypanosomosis Problem" August 2003, Vienna, Austria.

The technical feasibility of creating tsetse free zones was demonstrated a few vears ago on the island of Zanzibar, which—as repeated entomological, parasitological and serological surveys confirmed-is "accepted" to be free of tsetse flies and trypanosomosis transmission since 1997. The establishment of AU-PATTEC resulted in proposals on the creation of tsetse free zones in identified priority intervention areas (areas with high development potential for productive and sustainable agriculture and livestock systems).

Some aspects regarding proposals to create tsetse fly free zones on mainland Africa remain subject to controversial discussions. Among these is the question when and based on which criteria one can confidently assume and eventually declare an area free of tsetse flies.

It is difficult/impossible to "prove zero," i.e. the absence of tsetse flies or the disease they transmit. The various known data, play a role in decision making. including a) information relevant to the reproductive rate of tsetse under known environmental conditions (which would permit calculating the time required for a tsetse population to increase from its lowest possible level to a density at which it would be detectable by trapping); b) the known/assumed relative efficiency of different traps to

capture certain tsetse fly species; c) other known parameters like trapping duration and intensity; and d) information on the disease incidence and experience in epidemiology (parasitological and serological surveys as a key component of indirect sampling for presence of the tsetse vectors).

A meeting of international consultants was held at IAEA in Vienna, Austria, from 25-29 August 2003 for proposing action for a standardized decision process that will eventually permit declaring areas free of tsetse and the disease they transmit.

Using the OIE established phased procedure for declaring areas free of, for example Rinderpest, entomological and serological activities were identified that need to be conducted: a) prior to the intervention against tsetse and trypanosomosis; b) during the ongoing intervention campaign and c) two phases after completion of the operations ("assumption of provisional freedom" and "declaration of freedom"). The intensity of and duration of the respective monitoring and sampling for tsetse presence and disease presence/ absence differs in the respective phases based on statistic probability models.

The findings were outlined at the 9<sup>th</sup> meeting of the PAAT Advisory Group (PAG) in Pretoria, South Africa, 24-25 September 2003. PAG welcomed the

FAO/IAEA initiative and proposed to use the PAAT-L (the e-mail link of

PAAT) for international scientific review of the consultants meeting report.

#### Consultants Meeting on "Improving Sterile Male Performance in Fruit Fly SIT Programmes" 20-24 October 2003, Vienna, Austria

Integrated application of the sterile insect technique (SIT) against fruit fly pests is gaining momentum, with active programmes targeting ten species in the Americas, Europe, Middle East, Asia, Africa and Australia. Accordingly, several billion sterile male insects are being mass reared and irradiated in factories around the world, shipped (as pupae) to their destination, or in emergence facilities, and then released into the field, where they are expected to copulate wild females. Thus an industrial consisting process. of numerous complex steps, must ultimately achieve a biological goal. The exigencies of the industrial process often affect the biological qualities of the final product, and although a number of quality control and quality assurance protocols have been developed to ensure that the released flies are of the highest quality, high ratios of sterile to wild males are needed for the technique to be effective. Indeed, there is a general consensus that the SIT is an evolving science, and much can still be done to improve the efficacy of the technique.

Recent research has identified a critical period in the process during which the sterile males may be manipulated in a manner that will significantly improve their copulatory success following release. This is the period that begins with the arrival of pupae at the emergence facility and ends with their release in the field. The breakpoint of the late pupal stage is useful both conceptually and practically. This postemergence period includes the shipment of late stage pupae, pupal and fly emergence handling procedures at the field distribution sites, and the release of sterile flies into target field the populations. During this period. manipulation is possible of: a) the abiotic environment; b) the biotic environment. to include hormonal. semiochemical nutritional and supplements; and c) the release methods.

The Consultants Meeting recommendations were the following:

- a) Given the current demand for improving the cost-effectiveness of current SIT application against major tephritid fruit fly pests of international importance, a CRP on improving sterile male performance is recommended.
- b) In the light of recent advances in Dipteran physiology, chemical ecology and behaviour, the consultants identified the pre-release period as one that is amenable to manipulations that will significantly enhance sterile male performance.
- c) Research and development under the CRP should be directed toward an improved understanding of these processes in tephritid fruit flies and developing defined protocols for use in operational SIT programmes.
- d) The initial focus should be on the identified ten pest species of *Anastrepha*, *Bactrocera* and

*Ceratitis* that are currently the target of SIT action programmes.

- e) The selection of CRP contract and agreement holders should be based on a good balance between action programme personnel and scientists conducting basic research in physiology, chemical ecology, microbiology and behaviour.
- f) The establishment of a future separate CRP or individual contracts to target the important colonization, filter or mother colony management and strain selection issues, that will not be addressed by the proposed CRP.

#### Workshops and Co-ordination Meetings

# Workshop on "Tephritid Fruit Fly Identification," FAO/IAEA Laboratories, Seibersdorf, 4-8 August 2003

The workshop was attended by nine participants from Israel, Jordan and the Palestinian Territories, all of them personnel of the Plant Protection Authorities involved in the detection, monitoring and/or control operations of Tephritid fruit flies in their respective locations. After a general presentation on the taxonomy and importance of the Tephritidae, the participants received hands-on training and lectures on the *Dacini* (by Ian White) and the *Ceratitidini* (by Marc De Meyer) species of potential economic importance to the

Middle East region. One session of the workshop was dedicated to training on the differentiation between wild and sterile and mated/unmated medfly specimens (by Kevin Hoffman). The personnel trained gained basic knowledge in the taxonomy of Tephritid fruit fly species as well as in the respective identification of sterile and wild medfly adults to support the current medfly control projects, as well as the operations relevant to detection of exotic fruit fly species.

#### **Other Important News and Reports**

# Twenty-fifth Anniversary of the Moscamed and Tenth Anniversary of the Moscafrut Mass Rearing and Sterilization Facilities

The Mediterranean fruit fly mass rearing and sterilization facility, know as the "Metapa" Production Plant after the name of the small town where the facility is located in southern Mexico, and the *Anastrepha* production facility know as "Moscafrut," a short name for the Spanish term "moscas de la fruta," celebrated their **Twenty-fifth** and **Tenth Anniversary** of operations since they were build in 1978 and 1993, respectively.

The Metapa facility, with its average production of 500 million sterile flies per week, was the corner stone of the medfly eradication programme (Moscamed Programme) that the Mexico and USA governments initiated in 1977 and that concluded with the eradication of the pest from the state of Chiapas and from Mexico in 1982. Since 1982, sterile medflies produced in the Metapa facility together with sterile medflies produced in El Pino (the USDA facility located in Guatemala), have been the source of sterile flies for the 300km long and 100km wide sterile fly barrier which has for 20 years prevented wild medfly populations from spreading from the Guatemala and Mexico border region towards the interior of Mexico and the US.

This regional pogramme has been supported by the IAEA and FAO through Technical Cooperation Projects (TCPs) since its foundation in 1977. It was through one of these TCP's that the sterile insect technology (SIT) was transferred from the Joint FAO/IAEA Entomology Laboratories in Seibersdorf, Austria, to Mexico in 1978.

In the past 25 years, the Metapa factory has produced a total of 650 billion sterile medflies, the vast majority sterile male and female flies from a bisexual strain. Recently, in the past two years, the facility has been adapted for mass rearing of 300 million sterile males per week of a genetic sexing *tsl* only male strain. This is done by shipping eggs from El Pino facility in Guatemala to the Metapa facility in Chiapas. El Pino facility has had an only male *tsl* genetic sexing breeding strain for the past 7 years and is currently producing around 2.5 billion sterile male flies per week. This facility is the largest fruit fly mass rearing and sterilization facility in the world.

The Metapa facility, apart from being one important source of continuous supply of sterile medflies for the Moscamed Programme, has, in the past, supplied sterile medflies to the states of California and Florida, contributing to



the eradication of medfly outbreaks in these states.

In 1992, the Mexican Government decided to launch the Mexican National Fruit Fly Campaign aimed at controlling, through the use of SIT, endemic fruits flies of economic importance for the Mexican fruit industry. One of the first steps taken by the Campaign was the construction and operation of the Moscafrut mass rearing and sterilization facility, which started production in 1993. Since this year, the facility has been producing a weekly average of 150



million sterile Mexican fruit flies (*A. ludens*). Through the application of an integrated fruit fly management approach, including the release of sterile flies, it has been possible to develop and

maintain the States of Chihuahua, Sonora and California Baja (576,000km<sup>2</sup>) as fruit fly free areas in Northwest Mexico. From these areas fruits and vegetables are being now exported to the US without quarantine restrictions. Furthermore, through an agreement between the National Fruit Fly Campaign and the California Department of Food and Agriculture (CDFA), the facility supplies sterile Mexican fruit fly to the state of California to assist in Mexfly outbreak eradication efforts. The facility has the capacity to mass rear and sterilize West Indian fruit fly (A. obliqua), which is a key pest of mango in Mexico. A. obliqua free areas were developed in the southern tip of the Peninsula of Baja California and from the northern tip of the state of Sinaloa, where commercial mango is grown for exports, using an

integrated approach including the release of sterile flies.

As part of Anniversary celebrations the Mexican Government decided to dedicate the sterile insect production facilities to two persons that were instrumental in the efforts to control fruit flies in Mexico using an integrated approach based on the SIT. The Metapa mass rearing facility was named after Gutierrez Samperio. Jorge former Director General for Plant Protection in Mexico, and the Moscafrut facility after Dieter Enkerlin, Emeritus Professor of parasitology in Monterrey, Mexico and Plant Protection Advisor for the Mexican Government. The contributions that these two men have made in the field of plant protection in Mexico and Latin America will remain as a legacy for future generations.

# Control of Melon Fly (*Bactrocera cucurbitae* Diptera: Tephritidae) with Insecticidal Baits in Afghanistan

In Afghanistan in 1996, the country had been in crisis for 17 years, a civil war was continuing and, by this time, the infrastructure of the country was in ruins and the agriculture sector had not received support for development since the 1970's.

Melons are grown throughout much of Afghanistan. The melon fly is the key pest of this crop and it has a patchy distribution throughout the melon growing area and, where it occurs, is a problem for farmers. In Afghanistan in an uncontrolled outbreak, all the melons in a field can be infested with the larvae of this fly.

In 1996, the only control measure available to farmers from the bazaars were persistent, systemic insecticides such as Metasystox (demeton-S-methyl) and were often expired products. The use of such product gave poor control (around 60% in Herat province) and was assumed to result in fruits contaminated with insecticide residues.

Female flies are unable to develop eggs unless they feed on a protein rich diet. Between 1996 and 1998 the authors developed, in selected melon fields in Herat Province, Afghanistan, a method to control melon flies based on a homemade poisonous proteinaceos bait and in 1999 demonstrated its use to farmers. The bait of hydrolysed protein was made based on a meat broth prepared from camel or cow meat. Olfactory lures were added in the form of the smell of Cucurbitaceae (cucumber juice) and rotten protein (ammonia) prepared from fermented urine (the urine was latter replaced by 20 g of urea diluted in 500 ml of water because of rejection from village Mullah. farmers the and consumers). An insecticide (malathion 50WP and 100WP) was added so as not to mask the smell of the lures. The bait was then splashed around the vegetative parts of melon plants. The malathion was the preferred insecticide due to its availability, low vapour pressure, odourless, and due to it having no systematic activity thus with no risk of translocation into the fruit if applied to the leaves of the plant.

Baits were applied early in the morning by splashing with a brush. Maximum droplet size was about 10mm. The first application was made 7 July and repeated every 10 days (8 applications) until 18 September. Twenty fruits were chosen randomly and infested fruits counted. Results show that where no control takes place total crop loss is possible. If treatment starts during flowering stage, before fruit set, then better control is obtained than if the first treatment is applied when plants are already producing fruit. Baits containing 50g ai per liter gave as good control as those containing 100g ai per liter. Baits applied at distances of 10m gave as good control as spraying every fifth plant or sparying all the plants.

In order to reduce further the population of melon flies, field sanitation should be given high priority: destruction of unmarketable and infested fruit from the field by deep burial or feeding to cattle and the ploughing of fields to destroy pupae. It is important that every farmer in the area follows the same procedures to reduce the population of the melon fly. By the end of the 1998 season the results of field trials showed that the poisoned baits were giving a satisfactory level of control of the fly with less than 10% infested fruits in treated field compared with 90% in untreated fields.

Not all farmers were convinced that the treatment had any value for them, most likely because of the distribution of the fly, which is considered a bigger problem in some areas than in others. For some farmers the application of baits prepared in this way applied to food plants was a taboo.

The cost of preparation of three liters of bait is ca. US \$2.0 sufficient to conduct 25 to 30 applications to 0.2 hectare (1 jerib of land). Farmers made from six to nine applications over the season. Production is about 20 MT per hectare (4 MT per jirab) and melons are sold at about 16kg per dollar so the output is worth US \$1,250 per hectare (US \$250 per jirab). The value of this control measure to the farmer (90% of the crop) is, therefore, about US \$1,125 per hectare (225 per jerib).

Credits: This paper was written by Barry Stride<sup>1</sup>, Zaheer Habibi<sup>2</sup> and Gul Ahmad<sup>3</sup>. It was published in the November/December 2002 volume 44 number 6 of the International Pest Control Publication of CEPA and FAOPMA. The work was funded by UNDP and implemented as part of the FAO crops programme in Afghanistan.

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- 2. National Officer (Plant Protection), c/o FAO Sub-office, Herat, Afghanistan
- 3. Technical Assistant, c/o FAO Suboffice, Herat, Afghanistan.

# **The West Indian Fruit Fly (WIFF)** Anastrepha obliqua in Grenada: Initial Assessment for an Eradication Plan

The West Indian Fruit Fly (WIFF),



*Anastrepha obliqua,* was detected in the Caribbean Island of Grenada in April 2002, by the Pest Management Unit (PMU) of the Ministry of Agriculture, Lands, and Forestry and Fisheries. Just after the first fly find, a trapping network was established to assess the distribution of the fly in the country. Agriculture, although only 10% of the GDP, is still an important economic activity of the population.

Given the seriousness of the problem, the Ministry of Agriculture requested IICA for an expert mission to confirm the findings and recommend possible control options including eradication using the sterile insect technique (SIT). The main findings and recommendations were the following:

• Trapping data indicates that the WIFF is wide-spread in the island covering approximately 310 square kilometres.

- Based on the low counts in traps and fruit infestation levels the fruit fly appears to be in a colonization stage.
- Fruits of the genus *Spondias* (hog plums, red plums and yellow plums), which are considered to be the main hosts for this fruit fly species, are wide spread in the island. Guava trees can be found all over the island and infestations have been detected in this fruit in different areas. Availability of suitable hosts throughout the year and favourable climatic conditions will allow rapid increase and spread of the pest.
- Technically, eradication of the WIFF using the SIT is possible for the following reasons: 1) The conditions offered by an island in terms of reducing the risk of reinfestations is a major advantage; 2) an exotic pest still going through a colonization stage is unstable and can be more easily controlled; 3) population levels are still low which is an advantage. however. populations would need to be further suppressed in order for SIT to be effective; and 4) SIT technology for this fruit fly species is available and has been used in Mexico for eradication of this species from localized areas in the northwest part of the country.
- Eradication of WIFF from Grenada has political support and the Ministry of Agriculture has the basic infrastructure to carry out an

eradication programme in what is a relatively small infested area. The effective control of the pink mealybug using an area-wide biocontrol approach has set a positive precedent. This information was extracted from the report prepared by the IICA fruit fly expert (Dr. Aldo Malavasi) who visited Grenada in January 2003.

# Adaptation to Artificial Rearing During Successive Generations in the West Indian Sweetpotato Weevil *Euscepes postfasciatus* (Coleoptera: Curculionidae)

#### Shimoji, Y. and Miyatake, T.

Fruit Fly Eradication Project Office, Okinawa Prefectural Government, 123 Maji, Naha, Okinawa 902-0072, Japan.

Abstract: An eradication project has been initiated in the southeastern island of Japan to contain the West Indian sweetpotato weevil, *Euscepes postfasciatus*, using the sterile insect technique (SIT). This article reports on rearing this insect for over 14 successive generations on an artificial diet, an element required for successful use of SIT. The fecundity of reared weevils increased with each generation. Egg hatch (85-95%) and adult yield (3060%) were consistently high throughout the test period. Comparison of fecundity and pre-oviposition periods indicated that females from the artificially reared strain at generation 14 oviposited more and earlier in life than those from the base stock. These difference resulted from an increase in frequency (100%) of females that laid eggs without standard oviposition substrate in the artificially reared strain. Adaptation to an artificial diet is discussed in relation to the oviposition behaviour of *E. postfasciatus* and the success of SIT against this weevil.

Paper available in: *Annals of the Entomological Society of America*. 95(6): 735-739.



#### **MEETINGS, SYMPOSIA, CONFERENCES**

# Integration of SIT and Other Environment Friendly Methods for the Management of Key Citrus Insect Pests, Agadir, Morocco, 17 February 2004

A workshop entitled "Integration of SIT and Other Environment Friendly Methods for the Management of Key Citrus Insect Pests" will be held by the IAEA in Agadir, Morocco, on February  $17^{\text{th}}$ , 2004, as part of the 10th International Citrus Congress (February 15-20, 2004). Through lectures and discussion, the workshop will aim at addressing the use of the SIT as an IPM tool for area-wide suppression of insect pests. Among others, the progress of some SIT-based medfly control programs, the research done in various fields like trapping and medfly ecology, and the use of complementary methods within an area-wide suppression strategy will be discussed. Some of the presentations will also address the recent developments in the potential use of the



 $F_1$  sterility for the suppression of the false codling moth, a major pest of citrus.

#### A five-day Workshop on "Principles and Procedures for Rearing Quality Insects" Mississippi State University, 22-26 March 2004

The workshop is designed to meet the needs of insectary managers and other personnel involved with insect rearing, especially those aspiring to become rearing specialists.

This is the first university course of its type that focuses on the science and technology of insect rearing.

The five days of instruction will cover all major aspects of insect rearing. The instructors possess extensive scientific and practical experience in rearing phytophagous and entomophagous insects.

The course is offered by the Department of Entomology and Plant Pathology at Mississippi State University in partnership with the USDA-ARS Biological Control and Mass Rearing Research Unit. For more information, please contact:

Department of Entomology and Plant Pathology P.O. Box 9775 Mississippi State, MS 3972 Phone: +1 (662) 325-2085 Fax: +1 (662) 325-8837 Email: <u>bperrigin@enomology.msstate.edu</u> Website: <u>http://www.msstate.edu/Entomology/Re</u> <u>aringwksp.htm</u>

# Principles and Procedures for Rearing Quality Insects

March 22-26, 2004

Mississippi State University Starkville, Mississippi



## 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere. 16-21 May 2004, Ft. Lauderdale, Florida

#### Dear Colleague,

Mark your calendar and make plans to attend the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere scheduled 16-21 May 2004 in Ft. Lauderdale, Florida, USA. The web link has been provided below to keep you up-to-date on information regarding the conference and to assist you with making arrangements for your participation.

This working group meeting will provide numerous opportunities for participants share to up-to-date information and research, and to discuss common concerns with colleagues from the western hemisphere. This exchange of information is vital to the continued research and management activities to formulate new goals and approaches to management strategies and action programs for Mediterranean fruit flies as

well as other genera of pest tephritid fruit flies. We invite you to join us.

#### Who Should Attend:

The conference is designed to bring together entomologists, chemists, geneticists, biologists, taxonomists, consultants, managers and regulators who are actively involved in all aspects of tephritid fruit fly detection.

#### **Conference Objectives:**

In addition to presentations and discussions of current research and regulatory activities, the participants will develop a prioritized list of research needs. The improved communication and discussion among the participants that will advance the science related to the understanding and mitigation of exotic pest fruit flies that threaten agricultural prosperity. Participants will interact in an interdisciplinary setting to summarize and review the state-of-the-art in research and management activities and to formulate new goals and approaches to management strategies and action programs for the Mediterranean fruit flies as well as *Anastrepha*, *Bactrocera*, *Rhagoletis* and other genera of pest tephritid fruit flies.

#### **Call for Abstracts:**

All individuals involved in research and aspects of tephritid fruit fly detection, control and eradication programs are strongly encouraged to submit an abstract for consideration as a poster presentation. Abstracts must be submitted electronically by March 5, 2004 following the detailed submission instructions posted on the working group web site.

On-line registration and program information is posted on our conference web site

#### http://www.conference.ifas.ufl.edu/flies

Jennifer M. Anderson Conference Coordinator University of Florida/IFAS Office of Conferences & Institutes (OCI) PO Box 110750 Gainesville, FL 32611-0750 Phone: +1 (352) 392-5930 Fax: +1 (352) 392-9734 Email: jmanderson@ifas.ufl.edu http://www.conference.ifas.ufl.edu

# Workshop on SIT in Conjunction with the 15th International Plant Protection Congress, Beijing, China, 11-16 May 2004

A workshop on area-wide application of the Sterile Insect Technique (SIT) will be held in conjunction with the 15<sup>th</sup> International Plant Protection Congress in Beijing, China 11-16 May 2004. The First Great Gathering for Plant Protection in the 21<sup>st</sup>Century.

The Workshop will be part of the Section on Orchard Pest Management. Presentations on SIT projects will include: fruit flies in Thailand, Philippines, Australia and South Africa, red palm weevils in India, and sweet potato weevils in Japan. Also there will be a review of other insect pest species, especially moth species, against which there is a potential to apply the SIT.

IPPC Congress website: http://www.ipmchina.net/ippc/index.htm

## FAO/IAEA Interregional Training Course on "The Use of the Sterile Insect and Related Techniques for the Integrated Areawide Management of Insect Pests" University of Florida, Gainesville, Florida, USA, 4 May – 8 June 2004

Nominations should be submitted on the standard IAEA application form for training courses available on the web site: (<u>http://www-</u>

tc.iaea.org/tcweb/participation/astrainee/ default.asp). Completed forms should be endorsed by and returned through the official channels (Ministry of Foreign Affairs, Ministry of Agriculture, National Atomic Energy Authority, Office of the United Nations Development Programme or Office of the FAO). Nomination forms must be received by the IAEA, P.O. Box 100, A-1400, Vienna, Austria **not later than 5** 

### **OTHER ITEMS**

#### Trapping Guideline for Area-Wide Fruit Fly Programmes

During the 3<sup>rd</sup> Western Hemisphere Fruit Fly Workshop on Fruit Flies of Economic Importance, held July 1999 in Guatemala City, representatives of National Plant Protection Organizations (NPPO's) of 21 participating FAO and Member States IAEA expressed difficulties as a result of a lack of uniformity in the application of the various trapping methodologies to fruit flies of economic survey importance. They recognized the acute need for some harmonization of trapping procedures in view of the increasing fruit fly related trans-boundary interactions resulting from the rapidly growing travel, transport, tourism and trade. Thus they requested FAO and IAEA too develop some guidelines in support of their fruit fly survey activities for the various pest fruit flies.

These Trapping Guidelines for Fruit Flies of Economic Importance, developed in response to this request, provide strategic guidance and direction on where and how to implement surveys in support of fruit fly control and quarantine activities. This document is the summation of recommendations put forth by a multi-national group of fruit fly workers that has the goal of **January 2004.** A course prospectus is available upon request.

providing objective information on fruit fly survey tools to NPPO's and industry in FAO and IAEA Member States. These Trapping Guidelines are to be considered as a "working" document to regularly updated be as survey techniques continue to improve and experience in fruit fly control programmes evolves.

The guidelines are now available in hard copies upon request to the Insect Pest Control Subprogarmme and can be downloaded from the IPCS webpage <u>http://www.iaea.org/programmes/nafa/d</u><u>4/index.html</u>



# **Recent SIT Video Now Available in French and Spanish Versions**

The English teaching video "The Sterile Insect Technique. An environment friendly method of insect pest suppression and eradication" is now also available in Spanish (La Técnica del Insecto Estéril) and in French (La Technique de l'Insecte Stérile). Copies, in PAL, NTSC and SECAM formats, are available on request from the section. Contact one of the secretaries to obtain a free copy.

### Video on the Eradication of Tsetse from Zanzibar Available on the Web

Biting the fly—video on the success achieved in eradication of the tsetse fly from the island of Zanzibar. To see the video go to: <u>http://adminonline.iaea.org/videoclips/</u> <u>clip\_gallery.htm</u>



PUBLICATIONS

## **In Press**

- BAKRI, A.J. AND J. HENDRICHS.
  Radiation Dose for Sterilization and Disinfestation of Tephritid Fruit Flies.
  <u>In</u>: Proceedings of the 6<sup>th</sup> International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa.
- BENEDICT, M.Q. AND A.S. ROBINSON. The first releases of transgenic mosquitoes: An argument for the sterile insect technique. (*Trends in Parasit.*)
- CACERES, C., J. P. CAYOL, W. ENKERLIN, G. FRANZ, J. HENDRICHS AND A. S. ROBINSON. Comparison of Mediterranean Fruit Fly Bisexual and Genetic Sexing Strains: Development, Evaluation and Economics. <u>In</u>: Proceedings of the 6<sup>th</sup> International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa.
- CAYOL, J.P., Y. RÖSSLER, M. WEISS, M. BAHDOUSHEH, M. OMARI, M. HAMALAWI AND A. ALMUGHAYYAR. Fruit fly control and monitoring in the Near East: shared concern of a regional transboundary problem. <u>In</u>: Proceedings of the 6<sup>th</sup> International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa.
- CAYOL, J.P., W. ENKERLIN, A. BAKRI AND J. HENDRICHS. Les mouches des fruits (Diptera, Tephritidae): ravageurs d'importance économique pour l'agrumiculture. <u>In</u>: Proceedings of the CIRAD-CLAM Professional Meeting on "The Quality of Fresh and Processed Citrus Fruits: New Responses to the Expectations of Professionals". October 10-11, 2002, Montpellier, France.

- ENKERLIN W.R. AND M.M. QUINLAN. Development of an International Standard to Facilitate the Transboundary Shipment of Sterile Insects. <u>In</u>: Proceedings of the 6<sup>th</sup> International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa.
- NIYAZI, N., C. CACERES, A. DELPRAT, V. WORNOAYPORN, E. RAMIREZ SANTOS, G. FRANZ and A.S. ROBINSON (2003). Genetics and mating competitiveness of *Ceratitis capitata* (Diptera: Tephritidae) strains carrying the marker *Sergeant*, *Sr*<sup>2</sup>. (*Ann. Entomol. Soc. Am.*)
- ROBINSON, A.S. Mutations and their use in insect control. (*Mutation Res.*)
- ROBINSON, A.S. and P.W. ATKINSON. Insect transgenesis and its potential role in agriculture and human health. (Insect Biochem and Molec. Biol.)
- SUTANTAWONG, M., W. ORANKA-NOK, W.R. ENKERLIN, V. WORNOAYPORN AND C. CACERES. The Sterile Insect Technique for Control of the Oriental Fruit Fly, Bactrocera dorsalis (Hendel) in Mango Orchards of Ratchaburi Province, Thailand. In: Proceedings of  $6^{\text{th}}$ the International Fruit Flv Symposium, 6-10, 2002, May Stellenbosch, South Africa.
- VREYSEN, V, A.S. ROBINSON, J, HENDRICHS AND U. FELDMANN Activities of the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture in support of New and Old World Screwworm Eradication programmes. Proceedings International Screwworm Symposium, 28-29 August 2002, Tuxtla Gutierrez, Mexico.

2003

- ABILA P.P., KIENDREBEOGO M., MUTIKA G.N., PARKER, A.G. and A.S. ROBINSON (2003). The effect of age on the mating competitiveness of male *Glossina fuscipes fuscipes* and *G. palpalis palpalis. J. Insect Sci.* 3:13.
- BENEDICT, M.Q. and A.S. ROBINSON (2003). The first releases of transgenic mosquitoes: An argument for the sterile insect technique. *Trends in Parasit.* 19:349-355.
- ENKERLIN W., A. BAKRI, C. CACERES, J. P. CAYOL, A. DYCK, U. FELDMANN, G. FRANZ, A. PARKER. A. ROBINSON, M. VREYSEN AND J. HENDRICHS (2003). Insect Pest Intervention Using The Sterile Insect Technique: Current Status on Research and on Operational Programs in the World. In: "Recent Trends on Sterile Insect Technique and Area-Wide Integrated Pest Management Economic Feasibility, Control Projects, Farmer Organization and Bactrocera dorsalis Complex Control Study " Book published by Research Institute for Subtropics, Naha, Japan. 122 pp.
- ENKERLIN, W. (2003). Economics Of Area-Wide SIT Control Programs. In: Recent Trends on Sterile Insect Technique and Area-Wide Integrated Pest Management - Economic Feasibility, Control Projects, Farmer Organi-Bactrocera zation and dorsalis Book Complex Control Study.

### 2002

- CAYOL, J.P., P. CORONADO AND M. TAHER (2002). Sexual compatibility in medfly (Diptera: Tephritidae) from different origins. *Fla. Entomol.* 85(1): 51-57.
- CAYOL, J.P., J. HENDRICHS, W. ENKERLIN, A. DYCK, AND M. VERYSEN (2002). The Sterile Insect Technique: an environment friendly

published by Research Institute for Subtropics, Naha, Japan. 122 pp.

- HENDRICHS, J. and A.S. ROBINSON (2003). Sterile Insect Technique. <u>In</u>: V.H. Resh & R.T. Carde, (eds.). Encyclopedia of Entomology, p. 1074-1079 Academic Press/Elsevier Science, USA.
- IAEA (2003a). Trapping guidelines for area-wide fruit fly programmes. Vienna: IAEA.
- IAEA (2003b). Improved attractants for enhancing tsetse fly suppression: Final report of a co-ordinated research project 1996-2002. IAEA-TECDOC-1373. Vienna: IAEA. September 2003.
- IAEA (2003c). Automation for tsetse mass rearing for use in sterile insect technique programmes: Final report of a co-ordinated research project 1995-2001. IAEA-TECDOC-1353. Vienna: IAEA. May 2003.
- QUINLAN, M. M. AND W. ENKERLIN (2003). The Commercialization of SIT.
  <u>In</u>: Recent Trends on Sterile Insect Technique and Area-Wide Integrated Pest Management Economic Feasibility, Control Projects, Farmer Organization and *Bactrocera dorsalis* Complex Control Study. Book published by Research Institute for Subtropics, Naha, Japan. 122 pp.

method for the area-wide integrated management of insect pests of economic significance. <u>In</u>: Proceedings of the 2<sup>nd</sup> International Conference on the alternative control methods against plant pests and diseases. 4-7 March 2002, Lille, France, p. 593-600.

FAY, H.A.C. AND V. WORNOAYPORN (2002). Inert reusable substrates as

potential replacements for wheat bran in larval diets for Mediterranean fruit fly, *Ceratitis capitata* (Weid.) (Dipt., Tephritidae). J. Appl. Ent 126:92-96.

- FRANZ G. (2002). Recombination between homologous autosomes in medfly *(Ceratitis capitata)* males: Type-1 recombination and the implication for the stability of genetic sexing strains. *Genetica*. 116:73-84.
- HENDRICHS, J., ROBINSON, A.S. CAYOL, J.P. AND W. ENKERLIN (2002). Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. *Fla. Entomol.* 85(1):1-13.
- HENDRICHS, J. AND A.S. ROBINSON (2002). Sterile Insect Technique. Encyclopedia of Entomology. USA: Elsevier.
- LUX, S.A., MUNYIRI, F.N. VILARDI,
  J.C. LIEDO, P. ECONO-MOPOULOS,
  A. HASSON, O. QUILICI, S. GAGGL,
  K. CAYOL, J.P. AND P. RENDON
  (2002). Consistency in courtship pattern among populations of medfly (Diptera: Tephritidae): comparisons among wild strains and strains mass-reared for SIT operations. *Fla. Entomol.* 85(1):113-125.
- OLET, P.A., OPIYO, E. AND A.S. ROBINSON (2002). Sexual receptivity and age in *Glossina pallidipes* Austen (Diptera: Glossinidae). J. Appl. Entomol. 126:1-5.
- MUTIKA, G. N., E. OPIYO AND A.S. ROBINSON (2002). Effect of low temperature treatment on male pupae and adults of *Glossina pallidipes* (Diptera:Glossinidae). *Entomol. Sci.* 5:209-214.
- ROBINSON, A.S., CAYOL J.P. AND J. HENDRICHS (2002). Recent findings on medfly sexual behavior: implications for SIT. *Fla. Entomol.* 85(1):171-181.

- ROBINSON, A.S. (2002) Mutations and their use in insect control. *Mut. Res.* 511:113-132.
- ROBINSON, A.S. (2000). Transgenic Medi-terranean fruit flies for the sterile insect technique. Proceedings of "The 7<sup>th</sup> International Symposium on Biosafety of Transgenic Organisms" Beijing. 10-16 October 2002. p. 195-210.
- RODRIGUERO, M.S., VERA, M.T., RIAL, E., CAYOL, J.P, AND J.C. VILARDI (2002). Sexual selection on multivariate phenotype in wild and mass-reared *Ceratitis capitata* (Diptera: Tephritidae). *Heredity*, 89:480-487.
- RODRIGUERO, M.S., VILARDI, J.C. VERA, M.T. CAYOL, J.P. AND E. RIAL (2002). Morphometric traits and sexual selection in medfly (Diptera: Tephritidae) under field cage conditions. 85(1):143-149.
- ROBINSON, A.S. (2002). Introduction. *Genetica*. 116:1-3.
- ROBINSON, A.S. (2002). Genetic sexing strains in medfly, *Ceratitis capitata*, sterile insect technique field programmes. *Genetica*. 116:5-13.
- TODD, E.S., ROBINSON, A.S. CACERES, C. WORNOAYPORN, V. AND A. ISLAM (2002). Exposure to ginger root oil enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae) from a genetic sexing strain. *Florida Ent.* 85:440-445.

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