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Tsetse flies and the disease trypanosomosis they transmit, are responsible for the separation of livestock keeping and crop production in much of sub-Saharan Africa. As a result, productive mixed crop-livestock farming as shown above is still a rare exception in African agricultural practices. Insert: sterile male tsetse fly mating with a virgin female.

Contents

To Our Readers

In October 2004 the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture celebrated 40 years of existence. The creation in October 1964 of this Division, which includes the Insect Pest Control Subprogramme, marked the beginning of what is certainly a unique and arguably the best example of inter-agency cooperation within the whole UN family. The goal was to join the talents and resources of both organizations to obtain better cooperation and less duplication of efforts in assisting their Member States in applying nuclear techniques for providing people with more, better and safer food and other agricultural products, while sustaining the natural resources base. The complete press release is included under "Special News and Reports".

In June 1998 FAO and IAEA sponsored the **First International Conference on Area-Wide Control of Insect Pests Integrating the Sterile Insect and Related Nuclear and other Techniques** in Penang, Malaysia with almost 300 participants from 63 Member States and five international organizations. This Conference -

IAEA International Atomic Energy Agency

greatly increased awareness concerning the area-wide approach for insect pest manage ment programmes, some of which effectively integrate the SIT with other control methods.

Preparations for a follow-up **Second International Conference on "Area-Wide Insect Pest Control: Integrating the Sterile Insect and Related Nuclear and Other Techniques"**, sponsored by FAO/IAEA to be held in Vienna during **May 9-13 2005**, are well under way. Announcement available at:
http://www- pub.iaea.org/MTCD/Meetings /Announcements.asp?ConfID=131

So far the response has been excellent, and many interesting proposals have already been received from over 40 countries for oral and poster presentations, as well as for exhibits by diverse companies from the public and private sector. A committee will review all proposals to develop an interesting scientific programme that will include a number of very relevant invited keynote speakers from major non-SIT area-wide programmes in the field of agriculture and public health, to which the same area-wide principles and issues apply. Hence the programme will differ significantly from the conference in Malaysia, addressing not only other pest insects and area-wide programmes, but also focusing on non-technical issues that often determine success or failure of area-wide programmes. In view of the increasing interest and developments shown by the private sector, there will also be sessions on commercialization of SIT and regulatory issues. The Second International Conference will have as its theme the "Effective Implementation of Operational Area-Wide Programmes".

Looking back on the year 2004, it has once more been a busy time for all of us working at the Entomology Unit and the Insect Pest Control Section of the Joint FAO/IAEA Agriculture Programme. As you can see from the content of this newsletter, the team has been involved in many projects and there are a number of interesting activities to report.

One such activity was the preparation of the first comprehensive textbook on the SIT and this very timely project (in view that the SIT will soon cover a 50-year history) has turned out to be larger than originally foreseen. It reviews the various components of the technology from a generic point of view, rather than as collections of descriptions of ongoing SIT programmes, which can be found in a number of proceedings. This project is nearing completion, with close to 30 chapters written by experts in the field of SIT that have undergone critical peer-reviews. The copy-ready manuscript will be submitted to Springer for publication in 2005.

Moving to R&D activities, the Coordinated Research Project (CRP) on "Quality Assurance of Mass Produced and Released Fruit Flies" was concluded during a final RCM in Tapachula, Mexico, with the participation of 29 scientists from 17 countries. The final results of this six-year CRP (1999-2004) will be published in the Florida Entomologist, which is available as an open source journal often preferred by fruit fly workers for the publication of research articles.

As a result of this CRP, excellent progress was made in assisting fruit fly mass rearing facilities and end users to increase the efficiency and quality of production of sterile fruit flies through the standardization and improvement of quality assurance procedures. Two major outputs can be identified: (a) harmonized and improved QA/QC processes, data management, and information exchange on a global level, and (b) adoption of the quality assurance approach to varying degrees in most fruit fly SIT programmes world-wide. Specifically, the international quality control manual of standard operating procedures and minimum acceptable standards for QC of mass reared fruit flies was significantly updated with new and/or improved QC tests and new methods and procedures, including collection, analysis and information exchange of fruit fly quality control data.

At the same time we had the successful start of the new CRP D.4.10.20 on "Improving Sterile Male Performance in Fruit Fly SIT Programmes". The overall objective of this new CRP, approved for the period 2004-2009, is to reduce the cost and increase the effectiveness of SIT programmes by improving the performance of sterile males, specifically through hormonal, nutritional and semiochemical manipulations of sterile fruit fly males at the emergence and release facilities. Thus, the focus of this new CRP is the period from the moment the mass produced and sterilized late pupal stage leaves the mass rearing facility all the way through to field release.

Thirty scientists from sixteen countries participated in the 1st RCM, held in Antigua, Guatemala. Participants agreed to focus research during the next six years on four major interacting areas with potential to improve the performance of sterile fruit fly males: a) hormonal supplements, b) nutritional supplements, c) semiochemical supplements, and d) fly emergence, holding and release conditions. The CRP will focus on the ten tephritid fruit fly species currently the target of operational SIT programmes.

Finally I would like to call attention to a new CRP D4.10.21 on "Development of Mass Rearing for New World (*Anastrepha*) and Asian (*Bactrocera*) Fruit Flies", which was recommended by a Consultants Meeting in September 2002 and has now been approved for the period 2005-2010. This new CRP will focus on the development and improvement of mass rearing technologies for species of economic importance (mainly *Anastrepha* and *Bactrocera,* but also *Dacus ciliatus* and some *Ceratitis* ssp. from Africa) that cannot currently be reared in numbers sufficient to carry out area-wide SIT pro-

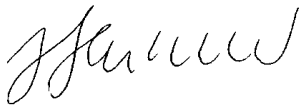
grammes. The objective is to bring rearing technologies for these species to a level where a decision can be made as to their suitability for use in fruit fly control programmes involving the SIT. Many research proposals have already been received for this new CRP, although the call for proposals is still open. The 1st RCM is tentatively scheduled for March 2005 in Quezon City in the Philippines.

In terms of the application of area-wide control and support to SIT field projects, we are starting the biennium 2005-2006 with a series of new technical cooperation projects that were recently approved by the IAEA Board of Governors, in addition to the continuation of a number of projects. However, the support to technical cooperation projects will be provided in the context of further re-orientation of the IAEA approach towards technical cooperation activities in support of Member State field programmes. This involves a shift in focus towards capacity building and technology transfer, rather than direct involvement in the implementation of operational field projects for which countries themselves have full ownership and responsibility.

As a result of this re-orientation and of various external and internal audits carried out this year on (tsetse) projects, we are now focusing on R&D, capacity building, development of manuals, operating procedures and e-learning modules, and technical and economic feasibility assessments. As the disappointing progress with the Jamaica New World screwworm eradication programme has shown (see section reporting on it), it is difficult to achieve eradication without adequate management structures and appropriate independent quality control mechanisms. It will therefore, be important to monitor the impact in the field of this re-orientation of TC projects.

On a more positive note, the application of SIT is nevertheless moving forward in other areas, with sterile insect rearing facilities currently being planned or built for fruit flies in Argentina, Brazil, Israel and Spain, and for New World screwworm in Panama, and the prospects for area-wide application against various moth pests is steadily improving.

On behalf of all of us at the Sub-programme, 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 your continued feedback and wish you a fruitful and very successful 2005.



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Forthcoming Events

I. Research Coordination Meetings (RCMs)

1. Development of Mass Rearing for New World (*Anastrepha*) and Asian (*Bactrocera*) Fruit Flies. 28 March–1st April 2005, Quezon City, Philippines. First RCM.

2. Developing of Product and Process Quality Control for Standadization of Tsetse Mass Production, Sterilization and SIT Production. 4-9 May 2005, Vienna, Austria. Second RCM.

3. Development of Improved Attractants and Their Integration into Fruit Fly SIT Management Programmes. 5-10 May 2005, Vienna, Austria. Fourth and Final RCM.

4. Evaluating the Use of Nuclear Techniques for the Colonization and Production of Natural Enemies of Agricultural Insect Pests. 12-17 May 2005, Vienna, Austria. Fourth and Final RCM.

5. Use of Molecular Tools to Improve the Effetiveness of SIT. 13-18 May 2005, Vienna, Austria. Second RCM.

6. Enabling Technologies for the Expansion of SIT for Old and New Screwworm. 30 May-3 June 2005, Montevideo, Uruguay. Third RCM.

7. RCM on Improvement of Codling Moth SIT to Facilitate Expansion of Field Application. 14-18 November 2005, Mendoza, Argentina. Second RCM.

II. Consultants and Other Planning Meetings

1. Ninth Meeting of the Programme Against African Trypanosomiasis (PAAT), 5-6 May 2005, Vienna, Austria.

2. Consultants Meeting on Handling and Release of Fruit Flies, September 2005, Vienna, Austria.

III. Other Meetings/Events

1. Second International Conference on Area-Wide Insect Pest Control, 9-13 May 2005, Vienna, Austria.

2. Seventh International Symposium on Fruit Flies of Economic Importance and Sixth Meeting of the Working Group on Fruit Flies of the Western Hemisphere, 10-15 September 2006, Salvador, Bahia, Brazil.

Past Events

I. Research Coordination Meetings (RCMs)

1. Quality assurance of mass produced and released fruit flies, 18-22 October 2004, Metapa de Dominguez, Chiapas, Mexico. Final RCM.

   Note: Reports available upon request

2. New CRP on Improving sterile male performance in fruit fly SIT programmes, 25-29 October 2004, Antigua, Guatemala. First RCM.

II. Consultants and Other Planning Meetings

1. Generic design and technical guidelines for the location of tsetse mass rearing facilities, 11-15 October 2004, Vienna, Austria.

2. Research planning meeting for mosquito activities in Northern Sudan in 2005, under project RAF/5/052. 25-26 November 2004, Vienna, Austria.

   Note: Reports available upon request.

3. 1st Steering committee meeting on mosquito monitoring activities for La Reunion Island to develop work plans for the coming years, 13-14 December 2004, Vienna, Austria.

4. Consultants meeting for development of CRP project proposal on mass production, handling, sterilization and field biology of adult *Anopheles arabiensis*, 15-17 December 2004, Vienna, Austria.

III. Other Meetings/Events

1. Cactus Moth (*Cactoblastis cactorum*) Regional Forum, 27-30 July 2004, Mexico City, Mexico.

2. Subregional Training Workshop on Managing Public Relations for Large-Scale Pest Control (Suppression and Eradication) Programmes using the Sterile Insect Technique. 30 August-1 September 2004, Guatemala City, Guatemala.

3. 10th Meeting of the Panel of the Programme Against African Trypanosomiasis Advisory Group (PAG) Coordinators, 22-23 September 2004, Accra, Ghana.

4. Fruit Fly Technical Panel Meeting under the Interim Commission of Phytosanitary Measures (ISPM) of the International Plant Protection Convention, Food and Agriculture Organization (IPPC/FAO). 20-24 September 2004, Bangkok, Thailand.

5. Subregional Training Workshop on Managerial Leadership and Strategic Planning for Large-Scale Pest Control (Supression and Eradication) Programmes that use the Sterile Insect Technique. 18-23 October 2004, Guatemala City, Guatemala.

6. Participation in the Technical Advisory Committee for review of eradication activities related to a Mediterranean fruit fly (medly) outbreak in Tijuana, 15-17 October 2004, Baja California, Mexico.

7. Participation in the Technical Advisory Committee for review of the Guatemala/Mexico/USA Moscamed Programme 21-23 October 2004 in Guatemala City, Guatemala.

Technical Cooperation Projects

For the new biennium 2005-2006, the Subprogramme will provide technical advice to the following on-going or new technical cooperation projects that are managed by the Technical Cooperation 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 Technical Cooperation Projects are:

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

BOT/5/002 Support of Tsetse Eradication from Ngamiland

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

ETH/5/012 Integrating SIT for Tsetse Eradication

INT/5/145 Promotion of Insect Pest Control 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/5/015 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

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

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

New Technical Cooperation Projects for 2005-2006 cycle are:

BGD/5/025 Studying the Feasibility of the Sterile Insect technique in Sun-dried Fish Industry Project.

BRA/5/057 Establishment of Medfly, Fruit Fly Parasitoids and Codling Moth Rearing Facility.

BKF/5/004 Feasibility Study on Applying the Sterile Insect Technique to Create a Tsetse-Free Zone.

CHI/5/047 Decresing the Population of the Mediterranean Fruit Fly in the Arica Region.

INT/5/149 Training Course on the Use of the Sterile Insect Technique and Related Techniques.

ISR/5/011 Strenghening the Capacity for the Area-Wide Control of the Mediterranean Fruit Fly Using the Sterile Insect Technique.

JOR/5/010 Strenghening the Capacity for the Area-wide suppression of the Mediterranean Fruit Fly Using the Sterile Insect Technique.

MALI/5/020 Feasibility Study for the Creation of a Zone Free of Tsetse.

MEX/5/029 National Prevention Campaign against the Cactus Moth

MOR/5/028 Assessing the Fesibility of Medfly Suppression Through the Sterile Insect Technique.

PAL/5/003 Strenghening the National Capacity for the Area-wide Suppression of the Mediterranean Fruit Fly.

PAK/5/043 Development of Biological Control for Cotton Pest Management Using Nuclear Techniques.

SAF/5/009 Preparation for the Creation of Zone Free of *G. brevipalpis* and *G. austeri*.

SEN/5/029 Feasibility Study to Create a Tsetse-Free Zone Free Using the Sterile Insect Technique

TUN/5/022 Implementation of the Pilot Programme Using Sterile Insect Technique against the Mediterranean fruit fly, Phase II.

URT/5/022 Assistance to a Feasibility Study for the Use of the Sterile Insect Technique.

In keeping with our policy to highlight activities in a few of our Technical Cooperation projects, the following projects are discussed in this issue:

The Jamaica Screwworm Eradication Project: (JAM/5/007)

The IPCS Newsletter of January 2003 reported on a change in the strategy to eliminate the New World Screwworm from Jamaica.

Figure 1: The fly distribution centre, where adults emerge from pupae kept in the central room and collected in the cold rooms left and right and from where they are transferred to the release containers

The new strategy, implemented since July 2002 called for a more focussed approach with activities concentrated in 3-4 parishes at the time and advancing from East to West in 6 distinct phases. During each of these phases, all animals were systematically inspected for infested wounds through house-to-house and farm-to-farm 'sweeps' and all wounds were treated with insecticides to continuously suppress the native fly population. Simultaneously, sterile flies were dispersed over the area under surveillance at extreme high densities. It was anticipated that this approach would result in the removal of the screwworm from the eastern half of Jamaica by May 2003. Despite encouraging results in the second half of 2002, this has not happened. On the contrary, the programme suffered from several serious set-backs in 2003 (see below), many beyond the control of the programme managers, which resulted in a record number of 469 reported screwworm cases in September 2003 (Fig. 3). Moreover, more than 40% of all monthly collected screwworm samples in 2003 contained third instar larvae indicating that many wounds were not inspected on time and that too many larvae were reaching full maturity.

The Government of Jamaica, in collaboration with the Mexico-US Commission for the Eradication of Screwworm initiated a large-scale experiment in early 2004, to test the performance of a new strain. The strain (JAM03) originated from biological material collected on Jamaica in 2003 and had been under development both at USDA-ARS facilities in Lincoln, USA and at the rearing facility in Mexico.

Figure 2: Release boxes with sterile screwworm flies being loaded into the release aircraft on Jamaica

The experiment was implemented according to the following protocol: 1) The collection of base-line data on screwworm infestation by treating the entire island with a uniform sterile fly dispersal rate with flies from the PAN95 strain (used for releases on the island since August 2001) for a period of six weeks. 2) An assessment of the mobility and longevity of sterile flies after release from the aircraft. 3) The dispersal of the JAM03 strain for 6-8 weeks over one-third of the island (the remainder of the island received sterile flies from the PAN95 strain). 4) An evaluation of the performance of the two strains based on the indicator 'reported number of positive screwworm cases'. The protocol did not include the collection of data on sterility levels induced in the native screwworm population, which would have given a direct indication of the mating frequency and the overall competitiveness of released flies of the two strains. The releases of the JAM03 strain were initiated in March 2004 over the Eastern parishes of Jamaica. The quality of the JAM03 strain, expressed in terms of laboratory parameters such as pupal weight, cross-over time in the dispersal facility, flight agility and emergence proved, however, to be highly unsatisfactorily and the dispersal of the strain was discontinued in July 2004.

Figure 3 shows that the proportion of infested cattle on the island declined from 0.25% in September 2003 to 0.15% in April 2004 but increased again to 0.21% in August 2004. The drastic decline in September 2004

(to 0.10%) might be attributed to the passing of Hurricane Ivan on 11 September 2004.

In June 2004, an independent team of experts, consisting of Mr Jimmy Bruce (retired USDA screwworm expert) and Prof. Aldo Malavasi (Professor Emeritus of Genetics, Sao Paulo University, Brazil and SIT expert) reviewed the programme. The team recommended that:

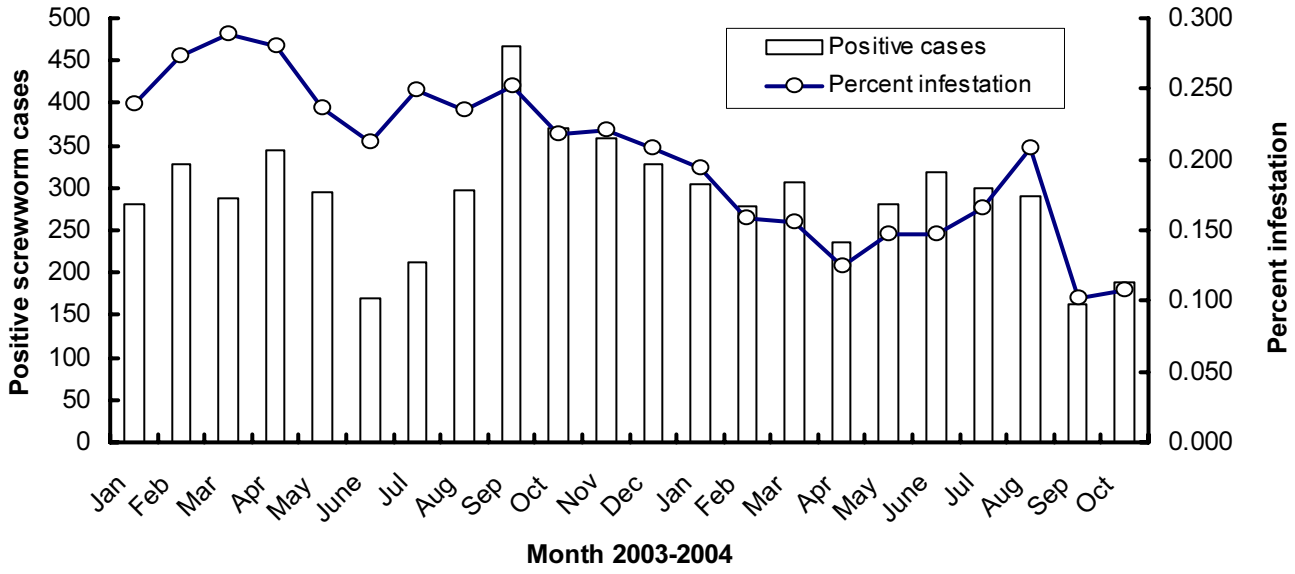

Figure 3 : The number of positive screwworm cases reported on Jamaica from January 2003 to October 2004 and the percent infestation (number of animals with infested wounds as proportion of total inspected)

1) The quality of the released insects should be improved to enhance their performance in the field. 2) A better management structure should be established to increase the overall efficiency of program implementation. 3) The field supervision should be improved to ensure a more efficient implementation of the field component. Following the review, improvements were made both in the fly dispersal centre in Jamaica and in the rearing facility in Mexico, which have resulted in better quality insects.

The screwworm eradication programme has faced many difficulties since releases of sterile insects were initiated in August 1999, and the following factors most certainly have contributed to the lack of significant progress:

Inconsistent programme implementation - Area-wide insect pest intervention programmes with an SIT component can only be successful if there is continuity in the implementation of all important programme components. From July 2002 (date of implementation of the new strategy) to December 2003, the programme has not seen any continuity in its operations for any period longer than 3 months. The situation in 2003 was maybe symptomatic with optimal implementation condition observed only in 25 weeks i.e. the programme suffered from the release of a batch of fertile flies (January 2003), the interruption in the releases or a reduced number of sterile pupae received (for a total of 10 weeks), a significant reduction in the quality of the sterile insects (3 weeks) and a reduction in the number of field inspectors employed (14 weeks). In view of the very short life cycle of the screwworm (21 days under optimal conditions), it is hardly surprising that little progress has been made considering the frequency of the interruptions.

Insufficient suppression of the screwworm population – Despite attempts to organise public awareness campaigns on the island, the programme has received little support from the animal owners in terms of the treatment of both infested and un-infested wounds. The very high density of host animals in Jamaica (i.e. 100-120 host animals per km^2) of which a significant amount (30%) does not have an owner (the so called 'stray animals') has exacerbated this problem. Despite the employment of 30 permanent and 20-25 temporary field staff (funded through IAEA TC Project JAM5007), the treatment of wounds has been insufficient to obtain adequate suppression of the native screwworm population on Jamaica.

Figure 4: Field staff inspecting a cow for the presence of wounds and screwworm larvae

Sub-optimal fly quality – The lack of systematically collected field data on mating frequencies of the sterile males makes it difficult to make sound statements on the quality and performance of the released insects. It is, however, significant that following the active surveillance campaigns (i.e. the 'sweeps') in the Eastern parishes during the second half of 2002, the number of screwworm cases dropped drastically and only a few relic pockets remained. The persistence of these screwworm pockets, despite extreme high sterile fly release densities over these areas, does seem to indicate sub-optimal performance of the sterile males.

Moreover, quality control/assurance tests carried out in the laboratory are important parameters that can ascertain inherent quality deficiencies as a result of the rearing. Figure 5 clearly indicates that the quality of the delivered sterile insects on Jamaica, expressed as average pupal weight, has significantly declined since the beginning of the programme in 1999.

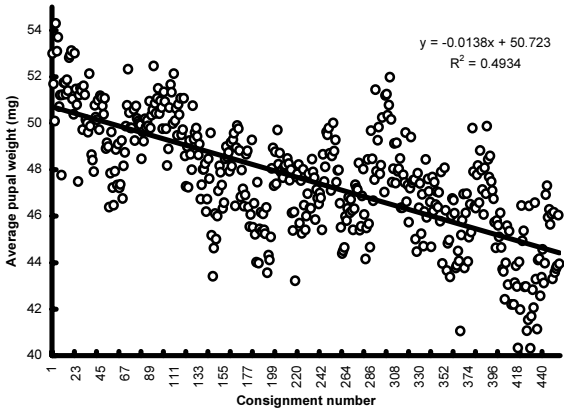

Fig 5: The average weight of the sterile pupae of each consignment received on Jamaica since the initiation of the programme in 1999.

The most crucial parameter to measure the efficacy of any SIT programme is the 'rate of induced sterility in the native population' (which is obviously related to the mating performance of the sterile male insects).

The need for the systematic collection of these data on the quality and competitiveness of the released flies in the field was pointed out on numerous occasions, but this was not given a practical follow-up until mid-2004. A total of 16, 37, 16 and 13 'egg masses' were collected in June, July, August and September 2004, respectively but sterility levels fluctuated only between 18.8% in August and 46% in September 2004, which is deemed insufficient to have any significant impact.

The future of the Jamaica screwworm eradication programme is at this stage uncertain although the Ministry of Agriculture of Jamaica has requested funds from the Ministry of Finance to continue with the operations in 2005. The support of the IAEA, through TC project JAM5/007 will however, come to an end in December 2004, which will entail that the programme cannot rely anymore on the crucial services of the International Advisor. It is extremely upsetting to contemplate a potential failure after 40 years of successful screwworm SIT programmes. The Jamaica programme is however, a plain and sobering illustration that 'success cannot be taken for granted'. Although most of the problems encountered in the programme were not related to the SIT technology *per se*, there are many lessons to be learned from this experience. Arguably the most important message would be that operational SIT programmes require 'an independent, flexible management structure that will implement the programme based on scientific principles, rather than following established operational procedures'.

Reality Check: Feasibility Studies for area-wide Tsetse Intervention in Mali (MLI/5/017)

The overall strategy of the Government of Mali to address their Tsetse and Trypanosomosis problem remains the creation of tsetse-free zones with the ultimate goal to eliminate the main tsetse species from their entire territory. Although many tsetse experts accept that control of the vector (i.e. the tsetse fly) remains the most desirable means of controlling the disease trypanosomosis, past experiences (e.g. the elimination of *G. pallidipes* from South Africa in the 1950's) have clearly demonstrated that sustainable tsetse-free zones can only be created if the intervention is targeted at an entire tsetse population within a circumscribed area i.e. the area-wide concept. Available tsetse distribution maps indicate that the tsetse belt is not continuous in many areas of East Africa, and this fragmentation could be exploited for the development of integrated control strategies.

The tsetse situation in West Africa is, unfortunately more complex as the distribution of most economically important tsetse species (the '*palpalis*' group) is closely associated with the riparian vegetation of the many river systems. The hydrological characteristics of any intervention area will therefore largely dictate the development, implementation and outcome of any control campaign. In that context, it has been postulated to use the concept of the 'river basin' as the unit of operation in area-wide integrated pest management (AW-IPM) campaigns. An important prerequisite for the implementation of this concept will be the isolation of the tsetse populations in the different, adjacent river basins (no immigration and emigration) with the watersheds between adjacent basins as efficient tsetse population barriers. Tsetse control activities in the past decades were mostly localised efforts, which were mainly implemented by the village communities and the isolation of the target tsetse population was often considered inconsequential. As a result, remarkable little research has been carried out on the dynamics, the ecology and the dispersal characteristics of riverine tsetse popula-

tions in West Africa. The urgent need for a feasibility study to assess whether sustainable tsetse-free zones can be created in West Africa in general and in Mali in particular has therefore become apparent. Some activities of this study have been supported under the TC project MLI5017.

1. Preliminary results of the feasibility study

The following activities have been carried out as part of the feasibility study: i.) a survey of the *G. palpalis gambiensis* (Gpg) population in the northern part of the Niger River Basin to re-assess their northern distribution limits, ii.) an analysis of the gene flow between Gpg populations of the Niger, Senegal and Bani Basins and iii.) a release-recapture study using sterile male and female flies to assess their dispersal potential in the watersheds between different river basins.

1.1. Northern distribution limit of the Gpg population

A survey zone with a total surface area of 9,195 km^2 was selected taking into consideration the distribution limits obtained during the last surveys of 1979 and 1989. The survey area was subdivided in different zones based on hydrological characteristics and potential survey points were identified using Landsat 7 imagery. Traps were deployed for 24 hours in a total of 745 sites and Gpg flies were sampled in 38 sites. The survey showed that i.) most of the flies were sampled in vegetation along the left bank of the Niger River ii.) flies were encountered in only one tributary of the left bank, iii.) the northern limit of the distribution seems to be situated more to the south as compared to the earlier surveys. The survey seems in addition to provide evidence in favour of the hypothetical model of Hendrickx et al. (Long term tsetse and trypanosomiasis management options in West Africa, PAAT Technical and Scientific Series, 6, 2004, pp 57), which postulates very fragmented riverine fly populations in this northern ecological zone.

1.2. The efficiency of the watersheds between river basins as natural population barriers

An analysis of the gene frequencies of the various Gpg populations was used to study the dispersal potential of Gpg flies belonging to populations of three river basins in Mali. (Fig. 6).

The DNA of 304 Gpg flies originating from the Niger River Basin, the Senegal Basin and the Bani Basin was extracted and gene frequencies tested among the different tsetse sub-populations with the premise that isolated populations are much more heterogeneous from each other than subpopulations which exchange flies. To test the homogeneity of the gene frequencies, mitochondrial DNA was chosen for various technical reasons. Only nine haplotypes were detected in Gpg (in contrast to 39 in *G. pallidipes*) and the mitochondrial diversity was low indicating that there must have been severe tsetse population reductions in the past. Very little genetic differentiation was observed between the sub-samples of the tributaries, which is indicative for a substantial rate of gene flow or exchange of flies between tributaries. The data therefore seem to indicate that Gpg populations belonging to these three different drainage systems in Mali are not well isolated. The exchange of genes possibly occurs during the rainy season, when the distribution of riverine fly populations is known to expand. The extent of this expansion, however, is not known and might be different for different areas.

It needs to be emphasized that the results from this research originate from a small fly sample from a limited geographical area in Mali. More systematic sampling covering the entire tsetse infested belt in Mali (and Burkina Faso) will be required to get a better understanding of the dispersal capacity of riverine species of tsetse in West Africa.

1.3 Release-recapture studies

Data from Burkina Faso, collected during the SIT programme in the Sidéradougou pastoral zone in the 1980's, already indicated the remarkable dispersal potential and mobility of Gpg. In one release experiment, 5 % of the released flies were recaptured at 21 km from the release point after only 5 days. In addition, Gpg flies have been recorded to cross from one river to another along a watershed of 6 km and fly across 4.5 km of forest clearings, and along 8 km of rivers with almost bare banks.

Fig. 6: Woody savannah vegetation in the watershed between tributaries of the Niger River Basin and the Bani River Basin (release – recapture study area)

In an earlier GIS study, 268 areas of potential contact between tributaries of the northern Niger River Basin and the Senegal and the Bani Basin had already been

identified. The width of the watershed was less than 4 km in more than seventy percent of these 'potential contact' areas. In view of the data available from Burkina Faso, this already indicated that there might be many potential areas of migration.

The release/recapture study was implemented to provide complementary data to the genetic study. Both sterile male and female Gpg flies, originating from the colony maintained at the CIRDES in Burkina Faso, were released in four release points in tributaries of the Senegal and the Bani Basin and an assessment was made of their dispersal over the watershed. The release sites were selected taking into account the presence and density of the vegetation, the distance from Bamako airport and accessibility.

The results indicate that, at least in one release site, the sterile flies were capable of crossing the watershed between the Niger and the Senegal Basin over a total distance of 3 km. It needs to be pointed out that no native flies were sampled during a survey of the area immediately prior to the release of the sterile insects. The existence or absence of native flies in the headwaters of the tributaries close to the watersheds is obviously an important factor. It is therefore planned to assess the dynamics of the native fly population in these areas as the temporal movement of the native flies along the tributaries will determine the period that the flies are present in the headwaters of these tributaries which will obviously influence the probability of crossing the watersheds.

The data of these studies have elucidated some of the earlier questions on the dispersal potential of flies belonging to populations of different watersheds and have confirmed the historical data of Burkina Faso. It is admitted that the collected data are limited in quantity and geographical scope but the results are sufficient to exercise extreme caution with the development of AW-IPM strategies.

2. Status on the suppression activities in the northern Niger River

Insecticide impregnated Vavoua traps (IIVT) have been used in the dry season of 2003 and 2004 to suppress the Gpg fly population in a first intervention zone (extended peri-urban area of Bamako) (Fig. 7). The deployment of the traps was carried out with the assistance of the local farmer communities. The suppression has so far not been implemented area-wide but the localised suppression of the Gpg population seems to be sufficient for the temporary alleviation of the trypanosomosis problem in this area. This was indeed corroborated by the entomological monitoring data (the Gpg fly population has been reduced drastically (e.g. only 6 flies were trapped in 52 traps (apparent density of 0.11 F/T/D) in the October 2004 monitoring on the left bank), the veterinary monitoring data (an average trypanosome prevalence of 0.9%) and the significant reduction in the use of trypanocidal drugs.

Fig. 7: Insecticide-impregnated Vavoua trap, used for the reduction of the Gpg population in the Niger River Basin in Mali

Taking into consideration the number of available personnel, the intervention zone will be expanded to include the Southern Niger River Basin (between the city of Bamako and the border of Guinea) and areas in the Bani River Basin located immediately east of the Niger River Basin. The suppression activities will not be repeated in the first intervention zone in the dry season of 2004-2005. Careful monthly monitoring of the fly population and a 'reactive approach' through the deployment of suppression traps in those areas where the fly population is found to be increasing, will be the strategy of choice of our Malian counterparts to consolidate the progress made in the first suppression zone.

Sterile Insect Technique for the Control of *Anopheles* Mosquitoes (RAF/5/052)

In collaboration with counterparts at the Tropical Medicine Research Institute (TMRI) and the Ministry of Health, Northern State of Sudan, research in selected parts of the project area has intensified. In both Dongola and Merowe, areas for intense surveillance of both larval and adult populations have been selected and stratification of land use completed. A grid system is used for randomly selecting blocks (100 x 100 m) in both areas, and these will be searched for breeding sites at monthly intervals. (Figs. 8 and 9). Using hand-held computers connected to a global positioning system (GPS), information on the locality and type of breeding sites, combined with details of mosquito breeding (species and density) and both physical and chemical characteristics of the sites, will be collected. In both field localities, houses are currently being rented to serve as field stations, and basic equipment has been provided. These will greatly facilitate the work in Northern State and provide an ideal base from which to operate. In

Khartoum, mosquito colonies from both localities are being maintained and used for research purposes.

Fig. 8: The Nile river, Northern State Sudan, source of water that breeds malaria vectors (photo: Bart Knols)

Initial DDT susceptibility tests have shown 100% knockdown/kill within 1 hr post-exposure. Further insecticide susceptibility tests are ongoing.

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

A contract was issued late in 2002 to the Institute of Zoology, Slovak Academy of Sciences, Bratislava (IZ-SAS), for "Provision of services for the mass rearing and supply of tsetse flies in support of area wide integrated intervention campaigns in tsetse and trypanosomosis affected Member States." Under the contract the IZ-SAS is required to prepare insectary facilities and develop tsetse colonies to supplement the capacity at Seibersdorf, so as to increase the available capacity to supply seed pupae to projects in Africa for their own colony development. Initiation of the work was delayed for administrative reasons, but the insectary facilities were completed and the colony initiated in mid 2003.

The staff of the IZ-SAS facility was trained in Seibersdorf, and we continue to monitor progress closely with regular visits to the institute by the technical officer. The colony initially progressed well, but heavy mortality was experienced in mid 2004, setting colony growth back by several months. A meeting was convened in Bratislava in August 2004 to review their rearing procedures, attended by the IZ-SAS staff and the technical staff from Seibersdorf. A number of recommendations were made, including changes to ventilation, fly handling and sterilization procedures. The recommendations were implemented, with immediate impact on the colony performance. Colony performance has now returned to normal, and the colony is growing according to expectation and now exceeds the total colony size in Seibersdorf. The target for the current contract is 100,000 females, which should be reached early in

Fig. 9: Fluctuation of the water level in the Nile leads to the establishment of breeding sites on its banks (photo: Colin Malcolm)

2005, although the insectary could hold in excess of 200,000 females.

IZ-SAS is currently holding three species of tsetse, *Glossina pallidipes*, *G. morsitans centralis* and *G. fuscipes fuscipes*, respectively required for TC field projects.

"A Medfly Rearing Facility in the Middle East" ISR/5/010

In early November 2004, at Kibbutz Sde Eliyahu in the vicinity of Bet She'an in Israel, a caterpillar started the first operations aiming at constructing the first private medfly rearing facility ever in the Middle East. (Fig. 10).

Fig. 10: A caterpillar starting first operations aiming at constructing the first private medfly rearing facility ever in the Middle East

The project, owned by Bio-Fly Ltd. (a sister company of Bio-Bee Ltd.), is two-phased. The first phase consists of the construction of a pilot facility capable of

producing about 15 million sterile male pupae per week by the first half of 2005. These pupae will address the present needs of the existing SIT operations in the Arava/Araba Valley of Israel and the Hashemite Kingdom of Jordan initiated in 1998. The second phase will consist of the construction of a large scale facility with a weekly production capacity of ±150,000 million sterile male pupae. During that phase, the initial pilot facility will be transformed to host the filter or mother colony. The production is intended to serve the needs of the SIT medfly suppression operations to be extended to the northern part of Israel, where part of the national fruit production is taking place, as well as to the northern part of the Jordan Valley, where over 90% of the citrus plantations of Jordan are located.

Throughout this project, Bio-Fly Ltd. will enjoy assistance and expertise on mass-rearing operations provided through the Plant Protection Inspection Services of the Ministry of Agriculture and Rural Development of Israel.

Status of Coordinated Research Projects (CRP's) and RCM's

Research Coordination Meeting on *Improving Sterile Male Performance in Fruit Fly SIT Programmes*, 25-29 October 2004, Antigua, Guatemala.

This new Coordinated Research Project (CRP) is based on a Consultants Meeting that was held from 18-22 October 2003 in Vienna, Austria (report available), to review progress made in this area, to assess the potential for conducting co-ordinated R&D, and to formulate a proposal for a CRP on "Improving Sterile Male Performance in Fruit Fly SIT Programmes".

The overall objective of this new CRP D.4.10.20, approved for the period 2004-2009**,** is to ultimately reduce the cost and increase the effectiveness of SIT programmes by improving the performance of sterile males in operational fruit fly SIT programmes, specifically through manipulations implemented at the emergence and release facilities.

Thus, the focus of this CRP is the developmental period from the late pupal stage of mass-produced fruit flies through to field release. R & D will focus on the ten tephritid fruit fly species currently the target of operational SIT programmes.

First Research Coordination Meeting (RCM)

Thirty scientists from 16 countries attended this first RCM, held in Antigua, Guatemala from 25-29 October 2004. The list of participants, which included all CRP contract and agreement holders, as well as a number of observers. (See picture of RCM participants, Fig. 11).

RCM participants presented ongoing research relevant to the CRP, as well as their research plans during the first half of the meeting. The agreed specific R & D objectives for the CRP are:

1. HORMONAL SUPPLEMENTS

For *Anastrepha spp., Bactrocera* spp. and *Ceratitis spp.*:

- Determination under standard conditions, if unknown, the age at which wild and sterile males become sexually mature.
- Assessment if JH treatment (or other conditions that stimulate JH production) improves significantly reproductive maturation, sexual behaviour and physiological parameters.
- Determination of the formulation and optimal dose required.
- Establishment of the application period and method required.
- Assessment of potential effect of irradiation on corpora allata.
- Assessment of interaction with irradiation, and nutritional and other supplements.
- Comparison of mating success of treated and untreated sterile males competing with wild males for wild females.
- Development of efficient large-scale methods for incorporation of JH analogues.
- Conduct of pilot scale tests that allow assessment of the method.
- Conduct of cost benefit analyses to determine feasibility of the method.

2. NUTRITIONAL SUPPLEMENTS

For *Anastrepha spp., Bactrocera* spp. and *Ceratitis spp.*:

- Conduct of basic research on adult diets and their effect on sexual performance of wild and sterile males.
- Characterization of the diet that optimises male sexual performance.
- Assessment of the interaction between diet, sexual performance, dispersal and survival.
- Identification of the optimal formulation and presentation in which diet is made available to sterile males, including the evaluation of inexpensive, locally available sources of nutrients.
- Study of the effects of mass rearing conditions and irradiation on gut physical and biochemical integrity.
- Assessment of the contribution of microorganisms to fly health and sexual performance, and investigate effects of inoculating sterile males with microorganisms on survival and copulatory success.
- Determinnation of a diet that provides the best cost effective balance between field performance and formulation.

3. SEMIOCHEMICAL SUPPLEMENTS

Anastrepha

- Search for semiochemical compounds that affect sexual performance.

- If such compounds are identified, proceed with objectives as specified for *Bactrocera* spp.

Bactrocera

- Assessment of various semiochemicals in terms of enhancing sterile male performance.
- Determination of optimal dose of ME/CUE that does not kill sterile male flies, increases their performance, and reduces their tendency to respond to MAT or ME/CUE monitoring traps.
- Determination of optimal feeding age, and time and duration of exposure, as well as the interactions between feeding on ME/CUE, hormone treatment and/or nutrition in terms of male competitiveness.
- Identification of a suitable standardized formulation to emulate a natural source of ME/CUE to achieve the desired mating competitiveness against wild males.
- Identification of an effective dispenser and commercial sources for ME/CUE.

Ceratitis

- Conduct of basic research on the behavioural, physiological and ecological effects of exposure to semiochemical supplements, such as citrus oils and ginger root oil.
- Identification of chemical composition, including active compounds involved, modes of action, and comparison of their effects.
- Development of standardized specifications, including comparison of different semiochemical sources.
- Identification the optimal formulation and protocols to make these substances available in SIT programmes.
- Identification of the optimal physical and biological conditions for incorporation into pre-release treatments for SIT programmes.
- Assessment of the cost-benefit of incorporating these supplements into SIT operations.

4. ABIOTIC ENVIRONMENT RELEASE METHODS

Fly Holding Conditions:

- Assessment of the effect of varying relevant environmental parameters, such as temperature, relative humidity, and illumination on sterile male performance.
- Comparison of the effects of different emergence systems, including varying the size (volume) of the containers and resulting fly density, on sterile male performance in the field.
- Assessment of the interaction of different holding conditions and hormonal, nutritional and / or semiochemical supplements, on sterile fly performance.
- Comparison of males resulting from standard conditions with males preconditioned to specific field conditions, such as high or low temperature, light intensity, elevation.

Fly Release Conditions:

- Assessment of the effects of cold knockdown for anaesthetising the flies on their performance, particularly for cold-sensitive species, and investigating alternatives if *required.*
- Assessment of effects on fly performance of different sterile fly release systems, as well as the interaction with hormonal, nutritional and / or semiochemical supplements.

Fig. 11. Group picture of RCM participants

Final RCM of the Coordinated Research Project on "Quality Assurance of Mass Produced and Released Fruit Flies for SIT Programmes" carried out in Tapachula, Chiapas, Mexico, 18-22 October 2004.

The overall objective of this CRP was to assist fruit fly facilities and end users to increase efficiency of production and use of sterile fruit flies through the standardization and improvement of quality assurance. The following specific research themes were addressed and results are planned to be published in an issue of the Florida Entomologist:

Colony improvement systems. Filter rearing systems (for GSS) or mother colonies (for bisexual strains) allow a systematic introduction of new strains or wild characteristics, respectively, and the study of different colonization methods. Field cage studies allow determination of strain quality in order to make decisions on the need for colony replacement or refreshment (introduction of wild genetically background). Studies of physiological adaptation of mass reared tephritids to variable outdoor conditions were also conducted.

Quality control and quality assurance in mass rearing. Production indices have been determined and quality parameters/standards developed and summarized in a manual. Recent research has shown that refreshment (hybridization) of mass reared colonies with wild or wild-type strains is likely to be better than replacement. Standard procedures and established threshold values have been summarized in the QC manual and there are species-specific differences and variation in the materials used. Production facilities are keeping records (based on standard procedures) of the quality of their production and field cage studies have been conducted and indices of performance published for some species, but still need to be developed for other species such as *B. tryoni, B philippinensis*. Large scale open field releases have led to accurate measurements of male performance in the field.

Nutrition. Laboratory studies have led to the development of chemically defined larval and adult diets but without field QC assessment. Gross diet manipulations affect the physiological traits of mass-reared males and facilities that use different larval and adult diets produce males of different quality. Recent results with chemically defined, and holigidic diets showed that pupating larval lipids and proteins can be affected by diet manipulations, but that regardless of diet history, emerging adult lipid levels tend to be regulated towards an optimum. Protein levels, in contrast, were more responsive to larval diet manipulations, and correlated with other measured QC-parameters. Protocols have been established for culturing and incorporating particular microflora into adult and larval medfly diets.

Dosimetry. A dosimetry system (Gafchromic system) has been developed and an SOP for the implementation of this system in SIT programmes has been compiled. The adoption of the Gafchromic system is in progress and training in Gafchromic dosimetry has been carried out with the result that some facilities are using the Gafchromic system for routine dose monitoring.

Day degree model of development. The number of day-degrees to reach the correct physiological age for irradiation of the bisexual strain of medfly has previously been calculated. Sufficient data is available to develop a matrix for use in other facilities. Some of this information is now available for the genetic sexing strains of medfly and for *B. philippensis*.

Long distance shipping. Studies on agitation, temperature and pupae packaging material have been carried out, but have not been satisfactorily completed. This also applies to the monitoring of long-distance shipping temperatures. A specific insulated pupal shipping container is already in use. Protocols have been developed on the shipment of eggs.

Field cage tests. The use of the mating performance field cage tests has become widespread in almost all mass rearing medfly facilities and has been extended to other fruit fly species. The QC manual has been revised and includes the copulation success test (CST).

Copula duration. Recent research on the medfly indicates that copula duration (CD) is not significantly linked to amounts of sperm transferred, nor to remating and therefore CD does not seem to be a reliable QA parameter in this species. Nevertheless, preliminary data on the role of CD in other species (e.g., *Anastrepha ludens*), suggests that it may be related to remating and further studies are indicated.

Female remating. In medfly females remate quite frequently. As many as 10% of wild females remate on the same day of the first copula under field cage conditions. Paternity studies suggest that as many as 30% of females in the wild utilize sperm from more than one male. Failure of sperm transfer by the first male has been shown to be the proximate reason for this behavior. It appears that copula duration, sperm transfer and secretions from the male accessory glands act in succession to curve female receptivity in this species. Female choice for the second mate is not significantly different from the first mating. In *Anastrepha ludens,* females whose first mate was sterile remate significantly faster and more frequently than females whose first mate was normal. Furthermore, results suggest that such females prefer a wild male as their second mate.

Sperm transfer and storage. Studies on the medfly indicate that sterile males transfer fewer sperm than wild males. Probability of sperm transfer and storage de-

creases as males age, suggesting that sterile males are most efficient during the 2 days following release.

Field survival. Knowledge has been developed which compares the relative field-cage survival abilities of sterile or normal laboratory flies vs wild flies. This has been conducted for several species of several genera, including *Bactrocera, Ceratitis* and *Anastrepha*. Information has also been obtained on the effects of nutritional resources (e.g. fruit, water) on survival under field-cage conditions.

Foraging. Sterile males are capable of foraging for food in the release environment. Pre release diet does not affect this ability.

Dispersal. Dispersal and longevity tests have been developed and validated but there is still a need for standardization of tests to assess survival and dispersal. The release recapture method has been adapted as a QC test and was used to evaluate the dispersal ability and survival of mass reared *A. ludens* and *A. obliqua* sterile flies in comparison with wild flies, sterile *B. philippinensis* of different sizes, bisexual and *tsl* strains of medfly V-7 and *B. cucurbitae* GSS and wild flies.

Coordinated Research Project (CRP) on: Enhancement of the Sterile Insect Technique through Genetic Transformation Using Nuclear Techniques.

This CRP concluded in 2002 with a Research Coordination Meeting held from 8-12 of July in Capri, Italy. The resulting articles have now been published in the journal Insect Biochemistry and Molecular Biology 34 (2004) 113-120, in a Special Issue entitled "Insect Transgenesis and its Potential Role in Agriculture". The papers published are listed in the table below.

There are three key areas in SIT programmes where the use of transgenic strains may be of considerable importance. First, the development of a genetic marker would remove the need to mark released flies with fluorescent dust. The current process is inefficient, expensive and has a negative impact on the sterile insects and on the health of workers in a mass rearing facility. Secondly, transgenic approaches to the production of male only progeny could improve the efficiency of current systems and would facilitate the transfer of the system to other pest insects. Thirdly, the creation of refractory strains, especially for tsetse, to support the use of SIT in areas of active human sleeping sickness transmission.

The CRP has played an important role in facilitating the research and exchange of ideas and reagents that has led to extraordinary progress in the area of transformation technology for non-drosophilid insects. This increased knowledge will provide the scientific basis that is absolutely essential for the successful evaluation of improved strains for the practical application in SIT programmes.

AUTHOR	PG.	TITLE
A.S. Robinson, G. Franz, P.W. Atkinson	113	Insect transgenesis and its potential role in agriculture and human health.
A.M. Handler	121	Understanding and improving transgene stability and expression in insects for SIT and conditional lethal release programmes.
M. Markaki, R.K. Craig, C. Savakis	131	Insect population control using female specific pro-drug activation.
L.M. Gomulski, C. Torti, V. Murelli, M. Bonizzoni, G. Gasperi, A.R. Malacrida	139	Medfly transposable elements: diversity, evolution, genomic impact and possible applications.
K. Komitopoulou, G.K. Christophides, K. Kolosaka, G. Chrysanthis. M.A. Theodoraki, C. Savakis, A. Zacharopoulou, A.C. Mintzas	149	Medfly promoters relevant to the sterile insect technique.
L.J. Douglas, P.M. Untalan, D.S. Haymer	159	Molecular sexing in the Mediterranean fruit fly, *Ceratitis capitata*.
K.A. Raphael, S. Whyard, D. Shearman, X. An, M. Frommer	167	*Bactrocera tryoni* and closely related pest tephritids-molecular analysis and prospects for transgenic control strategies.
C. Malva, P. Varricchio, P. Falabella, R. La Scaleia, F. Graziani, F. Pennacchio	177	Physiological and molecular interaction in the host parasitoid system *Heliothis virescens-Toxoneuron nigriceps*: current status and future perspectives.
M.J. Scott, J.C. Heinrich, X. Li	185	Progress towards the development of a transgenic strain of Australian sheep blowfly (*Lucilia cuprina*) suitable for male-only sterile release programme.

Fig. 12. List of papers published in "Insect Transgenesis and its Potential Role in Agriculture"

Developments at the Entomology Unit Seibersdorf

TSETSE

Installation of TPU3 in Seibersdorf

Work has started on the installation of the Tsetse Production Unit (TPU3) in the *Glossina pallidipes* colony area in Seibersdorf. The TPU3 is a semi-automated system for holding and feeding tsetse adults for colony maintenance that has been developed in Seibersdorf over the past four years to replace the former trolley holding system. TPU3 holds the fly cages in a frame that allows the flies to be fed without removing them from the holding frame. The feeding trays are placed on a rail-mounted trolley that runs along the row of holding frames. When the feeding tray is positioned under a set of cages the tray is raised allowing the flies to be fed, and after feeding moved on to the next set of cages. The pupae can be collected from the set of cages just fed whilst the next set is fed.

The TPU3 reduces the labour required for colony feeding. One or two technicians can feed a colony of more than 1 million females using the system. Equally as important is the fact that the system greatly reduces the disturbance to the flies caused by taking each cage off the former trolley system to place on the membrane and then returning it to the trolley after feeding. Any disturbance to the females produces stress, resulting in increased rates of abortion and higher female mortality.

The set to be installed in Seibersdorf consists of 7 standard holding frames, as there is insufficient space to accommodate a full set of 14 frames. The TPU3 will be used for training fellows and for testing the design to improve the ease of use and reduce the cost. Installation should be completed in January 2005.

Tsetse pupae sexing

As reported in our last newsletter, we have been conducting trials of a near infrared spectrometer for tsetse pupae sexing some days before emergence.

We have now placed an order for a near infrared (NIR) spectrometer and expect delivery in January or February 2005. The collaboration with Dr Floyd Dowell, USDA-ARS, Grain Marketing and Production Research Center, Manhattan, Kansas is continuing, and we hope to arrange another visit by him early next year to assist with the set up and calibration of the new machine. The new spectrometer will initially be tested for sexing *Glossina pallidipes*. If this proves reliable and easy to perform, we will continue to calibrate the system for our other tsetse species.

Sound recording

Under the CRP "Improved and harmonized quality control for expanded tsetse production, sterilization and field application" (D4.20.10) we have a research agreement with Prof H. Kratochvil of the Bioacoustics Section, Institut für Zoologie, Universität Wien. The agreement is to study the sounds produced by tsetse. Two students from the university are currently working on this project as interns at the Entomology Unit. They are looking at the pattern of sound production, including the frequency spectrum of the sounds, total energy, frequency of calling, and effects of time of day on calling. They have studied the effect of stress on sound production, and are comparing non-irradiated with irradiated flies. The objective of this work is two-fold: to try to identify those factors that are important in successful mating, and to find a general measure of "fitness" as a quality control parameter.

Developments in blood processing for tsetse diet

A number of new approaches to blood processing for tsetse diet are being investigated. Under the CRP mentioned above (D4.20.10) we are working with a contract holder to investigate pasteurization as an alternative to irradiation for reducing the bacterial load. Initial work in Seibersdorf had indicated that temperature/time combinations up to 72 ºC/10 seconds might not produce unacceptable increases in viscosity and the contract holder, using a trial scale pasteurising machine, is now following this up. He has confirmed successful heating to over 70 ºC, and this will be continued to higher temperatures to find when coagulation starts.

A second line is the use of citrate as an anticoagulant. A large batch of blood has now been collected successfully using citrate at about 0.015M; this will be tested on the colony on a larger scale to follow up earlier successful small-scale trials. A number of citrate anticoagulant mixtures developed from simple citrate are used in both human and animal medicine to store blood for later re-transfusion into the donor, and we are planning to investigate these to see if they improve the acceptance and nutritional quality of the blood. This work is being done in conjunction with the mosquito project, which also needs blood for diet and the various citrate derivatives will also be tested on mosquitoes.

Various additives to blood are also being investigated. The first group of additives are the phenothiazine vital dyes. These have been known since the 19th century to have anti-microbial activity, and are now frequently used in blood products in micro-molar quantities to prevent viral transmission. The possibility of these compounds being useful to restrict transmission of the salivary gland hypertrophy virus in the *Glossina pallidipes* colony will be investigated, but they should also be active against contaminant bacteria in the diet. Initial trials of four phenothiazines (methylene blue, dimethyl methylene blue, azure and neutral red) show that not only are they not toxic at active concentrations (1 – 10 μM) but the diet quality seems to improve, even though the trial was designed to demonstrate toxicity and not positive effects.

The second additives are nutritional components. Blood from different animals has differing nutritional quality for tsetse species. Generally tsetse will perform better on a mixture of cow and pig blood compared to either blood alone, and this has been attributed to limiting amino acid availability in pig blood and lipids in cow blood. Mixing them balances the amino acids and lipids. We therefore have been investigating lipid supplements for cow blood. The first trial was with pasturized liquid hen egg yolk. Unfortunately the egg yolk has a very high bacterial count, and even with 1 kGy gamma irradiation the load is still unacceptably high. Despite this 5% hen egg yolk achieved the same quality control factor value as unsupplemented blood. Tests will be continued with dried egg yolk products.

Remating in *G. pallidipes* and *G. brevipalpis*

In the previous newsletter it was reported that females that had a first mating with a sterilised male generally were unwilling to engage in a second mating 48 hours later. In a similar experiment, sexually mature female *G.pallidipes* were offered initial mating opportunities with unirradiated sexually mature males in a field cage in 1:1 male to female ratio. After separation the males were discarded and females returned to colony holding conditions. Two days later the females that mated with the unirradiated males were released in the field cage together with virgin sexually mature gamma sterilized males in a 1:1 male to female ratio. The observations in both cases lasted for two hours. The females mostly rejected attempts to copulate by males, with 0 - 10% accepting a second mating. Similarly as reported in the last newsletter, during the observation period in the field cage, it was noted that there was a very large number of rejections of second mating by females even with concerted efforts by the males. For the first mating, the females often accepted the male without a struggle. Dissections of the very few females that mated twice showed the presence of sperm in the spermathecae (from first copulation) as well as a mass of sperm in the uterus (from second copulation). Dissections of some of the females that mated with unirradiated males also showed that there were instances of incomplete filling of spermathecae among those females that rejected a second mating, partially indicating that incomplete spermathecal fill does not necessarily lead to remating. The levels of re-mating recorded in this restricted environment are unlikely to have significant effect on an SIT programme given that sterilized males will be released in numbers far larger than those present in the field.

Observations were initiated on the effect of multiple mating in *Glossina brevipalpis* with a mutation that phenotypically manifests itself as red eyes. The mutation is recessive and autosomal. Virgin sexually mature female red eye *G.brevipalpis* were initially mated with either normal eye or red eye males. A week later, the same females were mated with a red eye male if the first mating was with a normal eye male or normal eye male if the first mating was with a red eye male. The females did not readily accept a second mating thus the sample size was small. Pupae production was then followed for ninety days. Some of the females that mated with a red eye male followed by a normal eye male died early and the largest number of pupae produced by a single female was seven. Pupae produced were incubated and the progeny was scored. Emergence of a normal eye female when the parental cross was red eye female with red eye male followed by normal eye male indicated that sperm from the latest copulation were used for fertilisation. A larger sample size would provide results that are more conclusive.

FRUIT FLIES

Medfly genetic sexing

The productivity of several genetic sexing strains was analysed based on a large set of data that was collected during routine screening of these strains. In total ca. 115,000 eggs from 4 different sexing strains were analysed in comparison to a wild type strain. Because of their genetic structure, most sexing strains are 50% sterile, consequently the maximum number of offspring per 1,000 eggs is 250 males and 250 females at 25°C and only 250 males at the discriminating temperature of 34°C.

Four different treatments were applied to eggs: a) eggs collected for 24 h, without any temperature treatment (24h LL); b) eggs collected for 24 h, followed directly by a 24 h treatment at temperatures between 31 and 35°C (24h HL); c) eggs collected for 5 h, without any temperature treatment (5h LL); d) eggs collected for 5 h left for 24 h and then treated for 24 h at temperatures between 31 and 35°C (5h LH). Eggs were collected on wet filter paper that was then transferred to Petri dishes containing the larval diet. These closed Petri dishes were incubated at the temperatures indicated above.

Under these conditions the wild type strain produced on average between 839 and 903 adult offspring per 1000 eggs. Only the 24h HL treatment at elevated temperatures caused a significant reduction in productivity, e.g. at 34°C only 656 flies were found per 1000 eggs. In the case of the sexing strains, productivity was also affected by the egg collection regime even without temperature treatment. Collecting eggs for only 5 h increased the productivity on average by 25%, i.e. in case of the sexing strains VIENNA 4, 6 and 7 the 5h LL treatment produces on average 487 flies per 1000 eggs (however, in the strains VIENNA 4 and 6 this includes a large proportion of surviving adjacent-1 offspring). This is virtually identical to the expected value for a strain with 50% sterility. With a 5h LL treatment the strain VIENNA 8 produces 608 offspring per 1000 eggs, i.e. roughly 30% more than the other strains. This is the consequence of a different ratio of alternate and adjacent-1 segregation during male meiosis in this strain. The 24h HL treatment, for example at 34°C, leads to a complete elimination of the females but also to a significant reduction in male production. For example the strains VIENNA 4, 6 and 7 produce on average only 119 males per 1000 eggs and also the productivity of VIENNA 8 is reduced to 193 males. However, with the 5h LH treatment productivity is restored to the expected level; i.e. the strain VIENNA 6 produces 223 males per 1000 eggs (plus 54 adjacent-1 males), VIENNA 7 produces 201 males (without adjacent-1 males) and VIENNA 8 produces 279 males (without adjacent-1 males).

Mitochondrial analysis

The mitochondrial genome (mtDNA) is a very useful internal marker to discriminate wild flies from the release strain. In most cases four restriction enzymes (EcoRV, XbaI, MnlI and HaeIII) were used and were sufficient to obtain clear-cut conclusions. A simplified procedure, using these enzymes on PCR amplified fragments of the mtDNA, allows the screening of large numbers of samples without the need to do a Southern analysis. Samples from several locations, including for example South Africa, Madeira, Argentina, Tunisia and Guatemala were analyzed either with the goal to exclude contamination of the mass rearing strain or to determine whether an accidental release of fertile flies had occurred. An absolutely essential requirement is that the sexing strain carries only one mtDNA haplotypes and that this is different from the wild population in question. All sexing strains based on the wild type strain EgII carry such a mtDNA haplotype and that allowed discrimination from all wild samples tested so far.

Pupal irradiation atmosphere

David Nestel, a consultant from the Volcani Centre, Israel visited the Unit to conduct experiments to measure the quantity of oxygen inside of the plastic bags normally used for pupal irradiation. Pupae are placed in the plastic bags to reduce oxygen levels during radiation which in turn reduces somatic damage. The study was conducted with the VIENNA-8 strain and oxygen consumption was measured in sealed plastic bags containing 500 ml of male pupae. The pupae were 3, 2 and 1 days before adult emergence. It was shown that the more mature the pupae, the quicker the oxygen is used.

In a second set of experiments effects of irradiation temperature on fly quality were assessed. The pupae were exposed to a particular temperature for one 1 h before sealing the bag. The bags were then exposed to different temperatures and the oxygen consumption measured. When maximal hypoxia was attained inside the bag, the pupae were irradiated. Pre and post irradiation quality control tests were run. Result from the QC test and mating competitiveness field cage test have not shown any significant difference between treatments, but the control (no irradiation) has shown the higher quality values. Results suggest that pupae can be packed without any treatment to reduce metabolism. Pupae can be packed at room temperature and exposed to the irradiation treatment after one hour of having been packed. This procedure can represent savings in terms of energy, labour and time.

The third experiment determined the effect of the relative oxygen content in the sealed bag of pupae, on quality of the irradiated males. Pupae were irradiated under different oxygen environments of approximately 21 %, 10 %, 2 % and 0 % at the start of irradiation. Irradiation lasted for about 8.5 minutes. Results from the routine QC test and mating competitiveness field cage test have indicated no significant difference between hypoxia and partial hypoxia. However, irradiation in a normal atmosphere was clearly detrimental to fly quality. As already recommended, pupae should be packed at least one hour before irradiation to ensure sufficient hypoxia.

Fellowship training

Mr. Jaime Garcia de Oteyza was supported by the government of Valencia for a scientific visit to Seibersdorf Laboratories that will facilitate the establishment of an SIT medfly mass rearing factory against fruit flies in Spain. The visit covered medfly genetics and mass-rearing. Mr. Ferjani Dhaouadi was supported by the government of Tunisia for training on similar topics.

Rearing of *Anastrepha fraterculus* and *Bactrocera oleae*

Success has now been achieved in the establishment of a large colony of *Bactrocera oleae.* Improved oviposition devices and larval diet components have driven this development. Improvements have still to be made in the recovery of larvae from the diet, but the availability of this healthy colony will now enable large scale experimentation to be carried out. New egging cages are being

tested for *A. fraterculus* and new larval diets are being assessed.

MOSQUITOES

The second half of 2004 has seen a substantial increase in research activities on *Anopheles arabiensis*, the target species for development of mosquito SIT. The development of radiation protocols started in July and continues to focus on the selection of an appropriate life-stage and radiation dose, whilst aiming to safeguard the fitness and competitive ability of sterilised males. Trials, related to the development of a genetic sexing strain, have intensified since the arrival of state-of-the-art equipment in Seibersdorf. Research on blood sources other than human blood is ongoing and a promising combination of bovid blood and citrate might signal an important step forward towards the establishment of mass-rearing procedures. The Dongola colony of *An. arabiensis*, established in January 2004, is expected to replace the much older KGB colony (from Zimbabwe) before the end of this year, and will then become the sole target species for research. Field activities in Sudan have reached a stage whereby larval surveillance strategies in the Dongola and Merowe areas can be initiated at monthly intervals and training on the use of hand-held computers and GPS systems has been completed. Two field stations in each of these localities have been rented by the Sudan Ministry of Science and Technology and are being refurbished for research purposes. Two consultants, Ms. Michelle Helinski and Ms. Genevieve Labbe, have joined the project to focus on radiation biology and genetic sexing, respectively. Dr. Mark Benedict returned to the Unit in November to continue working in the project on a part-time basis.

Radiation biology of *Anopheles arabiensis*

Pupae, 20-26 hrs old, were irradiated in batches using the following doses: 0, 25, 35, 45, 50, 60, 70, 80, and 100 Gy. The effects of irradiation on pupal emergence, induced male and female sterility, and longevity and mating capability of the males was recorded. Although replicates of these experiments are still ongoing, it is clear that irradiation has no negative effect on the emergence rate of the pupae, which was similar to that of non-irradiated controls. Sterility was observed by mating the irradiated males and females to non-irradiated mosquitoes of the opposite sex. Mass oviposition in the cages occurred, after which fecundity and the hatching rate was determined. The egg production of irradiated females is almost completely inhibited at doses larger than 50 Gy, and of the few eggs that are laid, almost none will hatch. For the irradiated males, 100 Gy induced 99% sterility, while 50 Gy induced 80% sterility (respectively 10-30% for two repeats). Irradiation does not influence male longevity, which was similar to control males. The mating capability of the irradiated males does appear to be negatively influenced by irradiation to some extent, although there is substantial variation between different replicates of the same dose. Considering the variation in sterility levels and mating capability of the males, at least three replicates per dose will be completed before a definite dose-sterility curve can be drawn and the impact of pupae irradiation on mating capability is determined. The same dose range will then be repeated for the irradiation of the adults. There are indications that irradiation of adults will have less impact on mosquito fitness, but this needs to be verified. In addition, competition experiments will be initiated to test the competitiveness of the irradiated males.

Genetic sexing

The molecular laboratory is now fully functional, equipped with a state of the art microinjection platform and a screening system for the identification of transformed mosquitoes. Genevieve Labbe, from the University of Lyon has recently joined the group in support of the molecular research. Current efforts are concentrated on achieving germline transformation of *Anopheles arabiensis,* a critical step required before testing of conditional sexing systems based on lethal genes can take place. Female egg laying behaviour has been closely studied in the laboratory. Conditions have been identified that allow the collection of eggs throughout the day for microinjection purposes.

Blood diet

Research on blood sources other than human blood is currently under investigation, in particular, the impact of various anti-coagulants and different types of blood sources and storage procedures on mosquito feeding response and fecundity. Equine blood was briefly tested, but resulted in low feeding response and fecundity. For now, our attention is focused on bovine blood; the expertise in handling this blood is widely available within the Entomology Unit as tsetse is reared on bovine blood. Moreover, bovine blood is more readily available, both here and in the African partner countries, than equine or porcine blood. Bovine blood was either defibrinated or stored in citrate and frozen down. Aliquots of this blood were used to feed mosquitoes aged 4-5 days. Defibrinated blood resulted in low feeding response and subsequently a low fecundity. However, citrated blood seemed to elicit a better response. More research with citrated blood is underway; in particular ways to increase the attractiveness of the blood by the addition of ATP.

Special News and Reports

Joint FAO/IAEA Programme
of Nuclear Techniques in Food and Agriculture

40th Anniversary of Founding of Joint FAO/IAEA Division

1st October 2004

Better cooperation, less duplication of effort and shared approaches to building a peaceful and prosperous world. Member Governments have made it clear that this is what they want from the agencies of the United Nations system.

The creation in October 1964 of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture marked the beginning of what is certainly a unique and arguably the best example of inter-agency cooperation within the whole UN family.

The aim of setting up the Joint Division was to use the talents and resources of both Organizations for broadening cooperation between their member countries in applying nuclear science and technology in a safe, secure and effective manner for providing their people with more, better and safer food and other agricultural products while sustaining natural resources. Its uniqueness stems both from the nature of the technology itself and from the fact that all activities for applying it to food and agriculture within the UN system are conceived, planned and executed jointly by IAEA, FAO and their Member States only after their scrutiny and approval by their Governing Bodies - implicitly providing a "nod of approval" from both the world's Ministries of Agriculture and the world's Atomic Energy Authorities.

Yet, however close and harmonious the cooperation and however sophisticated the technology, it would not amount to anything if the benefits were not felt by the people and countries themselves. Looking back over these 40 years of cooperation, there have been many real benefits. Some of the most notable and sustainable examples that come to mind are the millions of hectares of higher yielding or more disease resistant food and industrial crops grown in fields all over the world from using radiation-induced mutations; the huge savings in fertilizer applications made possible by using isotopes to more effectively determine optimal placement and timing of use or to let plants fix nitrogen from the atmosphere; the eradication of screwworm from Libya, fruit flies from several countries in Latin America and the tsetse fly from Zanzibar using the Sterile Insect Technique; and the widespread use of immunoassay technology now being made by countries in all regions to diagnose and progressively control transboundary animal diseases like rinderpest and foot-and- mouth.

But past performance is no guarantee of future success. Today, both FAO and IAEA strive to mobilize commitment and action for meeting the World Food Summit and Millennium Development Goals of reducing hunger, poverty and environmental degradation through sustainable agriculture and rural development. The Joint Division is an integral part of that effort, concentrating on monitoring advances in nuclear technology and related biotechnologies and fostering the production and sharing of knowledge, know-how and techniques among governments, scientists, farmers and all others connected with food supply chains where these hold potential for bringing value.

Globalization, developments in international and national law and policy and the entry of increasingly diverse actors such as NGOs into the social dimensions of science and decision-making on its applications are just some the factors that have made this task more challenging than ever before. It is evident that no matter how useful, these techniques and products do not by themselves provide answers to all questions or solutions to all problems. They are, nevertheless among the alternatives to be considered and integrated within the broader spectrum of science and traditional knowledge-based approaches. Some are controversial and not surprisingly societies' attitude towards them differ widely across countries depending on their views on where the balance lies between the risks and benefits of their use. It's therefore essential that national policies and legal and regulatory frameworks consistent with international standards are in place to assess and manage the risks if these applications are to be accepted for adoption in food and agriculture. But equally important to build consensus and concerted action, there must be a free flow of objective information as well as communication with all groups of society on all possible uses and consequences.

Much remains to be done to remove the scourge of hunger from our midst and empower the food and agricultural sector to play its critical role in promoting sustainable development. The Joint Division has responded well to meeting these challenges, helping countries adapt to both for the rapidly changing landscape of agriculture and the evolving conditions for generating and sharing

knowledge and technology derived from the nuclear and biological sciences while taking into account the underlying diversity of social and economic contexts.

Retaining scientific rigour, objectivity and balance were key factors in its success over these 40 years. Others were vigorously pursuing its original objective of ensuring that all the knowledge and experience within FAO, IAEA and the global agricultural community including non-government and civil society actors were brought progressively into the process of decision-making on the Joint Division's Programme. All these helped it move from "single issues" towards increasingly holistic approaches to understanding and responding to global, regional and national needs and setting its priorities accordingly. Building on this recipe will be the roadmap for further success in the future.

Guest Article:

Presence of *Cactoblastis cactorum* and potential risks to native *Opuntia* in Cuba

The Cactus Moth, *Cactoblastis cactorum* (Berg). (Lepidoptera: Pyralidae) is a potential risk to the diversity of Cuban and U.S. cactus pear species. However Mexican *Opuntia* are the most endangered since a higher number of native taxa are registered to this country.

The outstanding results of the use of *C. cactorum* as a biological control agent in Australia and South Africa led to its deliberate introduction into the eastern Caribbean islands in the 1950's to control some native cactus pear species. Thereafter, the intentional or natural introduction of the cactus moth was registered in the western Caribbean islands including Puerto Rico, Hispaniola (Haiti and Dominican Republic) and Cuba where it was first reported in Guantánamo in the 1980's.

Most recent studies on the distribution of the *C. cactorum* in Cuba refer to at least five confirmed spots along the main island and the Municipality of Isla de la Juventud (Figure 13), which raises the potential risk, not only to the native cactus pear flora but to all the *Opuntia* species in the region. On the other hand, the intensive activity of hurricanes against Cuban and Florida territories may increase the possibility of natural introduction of the cactus moth into Mexico.

According to recent pest risk analysis studies, *C. cactorum* potential host range in Cuba has been estimated to comprise at least eleven *Opuntia* species and to date official host records include *O. dillenii*, *O. ficus-indica*, *O. (=Nopalea) cochenillifera* and two unidentified taxa.

However, the most endangered species, considering their limited distribution in the island and growing characteristics, are *O. militaris* and *O. triacantha*.

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Figure. 13: C. cactorum *detection sites in Cuba since its first report in 1980*

Cactus Moth (*Cactoblastis cactorum*) Regional Forum

A Regional Forum on Cactus Moth (*C. cactorum*) was organized jointly by the International Atomic Energy Agency (IAEA) and the Plant Protection General Directorate of the Government of Mexico (DGSV/SENASICA). The forum was held in Mexico City from 27 to 20 of July 2004. Participants to this important Forum included scientist from Florida, USA, Mexico and South Africa, as well as Mexican associated growers and representatives of the prickly pear industry, plant protection officials of the United States Department of Agriculture and of the Mexican Plant Protection Directorate, officials from the Mexican Ministry of Environment, representatives of agriculture of relevant states, the North American Plant Protection Organization (NAPPO) Technical Director and representatives of non governmental organizations.

During the Forum the current status of research in developing a cactus moth integrated pest management approach with a sterile insect technique (SIT) component was discussed. A basic package to control this insect pest through the area-wide application of the SIT has been developed by US and South African scientists with support from the FAO/IAEA. This package is now available and includes the following: low impact insecticides that

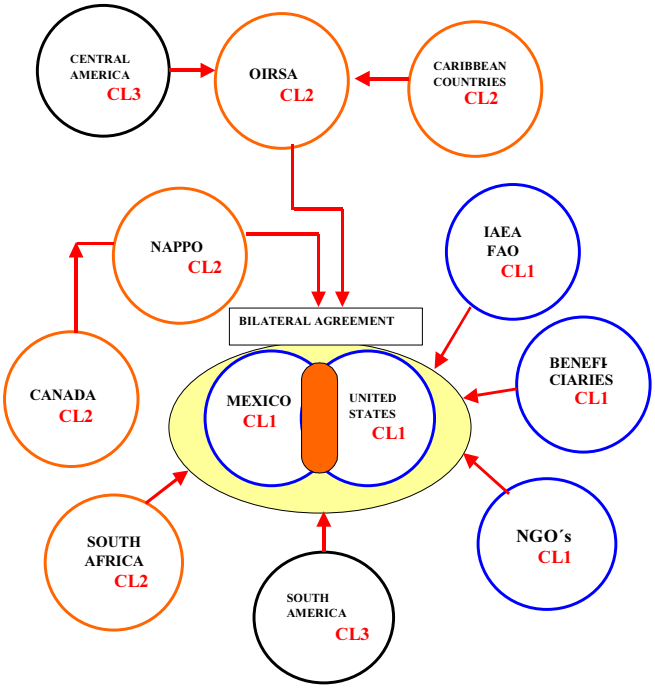


Fig. 14: Proposed strategic integration of relevant regions, countries and organizations

need to be registered for use against this pest, the technique for effective artificial mass rearing and sterilization, and a sex pheromone for monitoring and detection purposes. An update on the current leading edge of the infestation was presented by the US scientists. The pest has now been detected in the State of Alabama near the limits with Mississipi State and advancing at an estimated rate of 158 km per year. At this rate the pest would reach Texas by 2007.

If this occurs nothing would stop the spread of the pest to the South Western US States and into the vast *Opuntia* ecosystems in the arid regions of Mexico. The need for a regional proactive and preventive interinstitutional approach to the problem was also discussed and agreed upon. A regional strategy was proposed as follows:

The proposal identifies the countries, regions and the national and international organizations (governmental and nongovernmental) which should participate and integrate in a regional effort in order to face the problem in a more effective manner (see Fig. 14). The strategy proposes that Mexico and the United States, which are the countries that face an imminent risk of introduction and establishment of the pest, collaborate actively and closely through establishing a binational agreement (high collaboration level, CL1). The ideal would be to have an inter-regional collaboration with active participation of the Caribbean and Central American subregions. However, considering the priority that this problem has in these subregions and the prevailing conditions from a point of view of availability of financial resources and infrastructure to face this problem, the possibilities of establishing an inter-regional agreement are slim. Under this premise the Caribbean SubRegion would have a medium collaboration level of (CL2) through the existing plant protection infrastructure (see chart). Other subregions or countries (i.e. Central America and South America) would have a passive type of collaboration (low collaboration level, CL3) through *adhoc* mechanisms in specific cases as indicated in the chart. In the case of Canada the collaboration level would be CL2 through NAPPO and collaboration with the Caribbean Islands would be through OIRSA. South Africa would have a collaboration level of CL2 providing scientific and technical support to the project through binational negotiations or through the technical cooperation project that the IAEA has with Mexico. The scheme proposes an active participation of non-government organizations including the industry with a CL1 collaboration level contributing with efforts to create awareness among the general public and with financial, infrastructure and logistic support to confront the problem in areas of prickly pear commercial production. The IAEA would have a CL1 collaboration level contributing to the capacity building through the Technical Cooperation Project with Mexico and through providing research contracts.

Fruit Fly Technical Panel Meeting Under the Interim Commission of Phytosanitary Measures (ISPM) of the International Plant Protection Convention (IPPC)

Given the need to speedup the preparation of International Standards for Phytosanitary Measures (ISPM) in support of agricultural trade, the Interim Commission of Phytosanitary Measures (ICPM) of the International Plant Protection Convention (IPPC) has decided to create a number of Technical Panels to develop the required standards. The Technical Panel on Fruit Flies is the first Panel created under the ICPM indicating the impact that fruit fly pests have on international trade.

The first meeting of the Fruit Fly Technical Panel was held in Bangkok, Thailand, from 20 to 24 September 2004. The Panel participants drafted an International Standard (IS) for Fruit Fly Pests Free Areas (FF-PFA). The draft standard will be presented to the ICPM in December and then sent out for country consultation. The Panel also drafted the specifications for the IS on Fruit Fly Low Prevalence Areas (FF-PLPA). The specifications will be presented to the Standards Committee of the ICPM for approval. Once approved the specifications will come back to the Technical Panel for developing of the draft standard.

Announcements

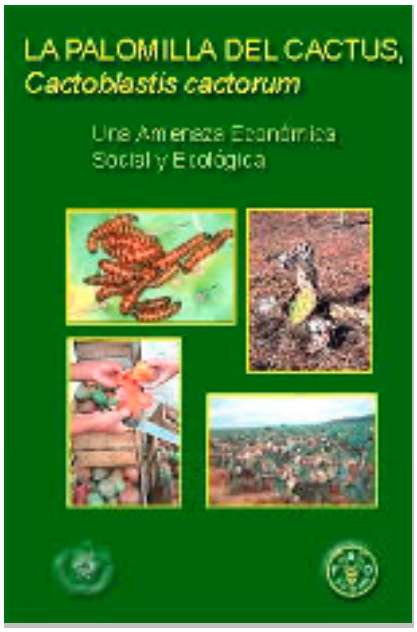
Second International Conference on Area-wide Insect Pest Control, Vienna, Austria 9 to 13 May 2005

This International Conference follows the one held in Penang, Malaysia from 28 May to 2 June 1998. The Conference will provide a forum for the presentation of scientific papers dealing with this topic and will include significant time for plenary discussion. The Conference will address the area-wide management of insects pests in general, as well as new developments and techniques in the field of SIT, F-1 sterility, genetics, biotechnology, mass rearing, ecology and behaviour, augmentative biological control, regulatory control and programme management. The Conference will be held in conjuction with a number of Research Coordination Meetings. Within the next few weeks the framework of the conference will be developed and the information will be placed on-line on the following web site for your consultation.

http://www-pub.iaea.org/MTCD/Meetings/Announcements.asp?ConfID=131

Cactus Moth (*Cactoblastis cactorum*) Video available in Spanish.

The video was prepared for a technical audience. It presents an overview of cactus moth biology and ecology and highlights the potential devastating economical, social and ecological effects to countries and regions of the world where *Opuntia* is considered to be a valuable resource.



New Book on the Sterile Insect Technique Available in 2005

The first textbook on the Sterile Insect Technique (SIT) will be published soon. The 29 chapters, written from a generic perspective by more than 50 authors from around the world, review all aspects of the SIT. The book is divided into eight sections: Introduction, Principles of the SIT, Technical Components of the SIT, Supportive Technologies to Improve the SIT, Economic, Environmental and Management Considerations, Application of the SIT, Impact of SIT Programmes, and Future Development of the SIT. When the book is published, information can be obtained from the publisher: Springer, Springer Science+Business Media, 3300 AA Dordrecht, The Netherlands.

Website:
http://www.springeronline.com/sgw/cda/frontpage/0,11855,5-10028-0-0-0,00.html

Information will also be available on the website of the Insect Pest Control Section:

http://www.iaea.org/programmes/nafa/d4/index.html

More information on this new book will be provided in the July 2005 edition of this newsletter.

Workshop on Insect Diets: Science and Technology

The Insect Diet and Rearing Institute, LLC, is a privately owned company dedicated to:

- The advancement of insect diet and rearing science and technology through education, consultation, and research.
- The centralization of diet and rearing related issues (acting as clearing house).
- The concept of continuous improvement.
- The Insect Diet and Rearing Institute, LLC, does not sell or supply insects or insect diets.

You can browse through the site (www.insectdiets.com) to learn more about what the Insect Diet and Rearing Institute, LLC.

Rearing of natural enemies

This Institute is announcing a new workshop for 2005 with Allen Cohen, the author of *Insect Diets: Science and Technology*, CRC Press.

Topic: Insect Diets and Rearing.

The course is based on the principles presented in Dr. Allen Carson Cohen's text.

When: February 21-25, 2005.

Where: The Insect Diets and Rearing Workshop will be offered at the Mesquite Room of the Student Union at the University of Arizona campus in Tucson, Arizona, USA.

Predatory insect in action

Special features:

One half day tour of the USDA, APHIS Pink Bollworm Rearing Facility in Phoenix, AZ.

Optional Natural History of Saguaro National Park Field Trip (8:00 am to 2:00 pm) on Saturday, February 26.

Learn directly from Dr. Allen C. Cohen:

- How diets work and why they sometimes fail to work.
- How to develop a data-based diet quality control system in your insectary.
- How to detect and prevent diet deterioration from microbial sources.
- How to detect and prevent other forms of diet deterioration (oxidative stresses, physical deterioration-such as separation of components-failure of gelling agents, and many other common diet problems).
- How the insectary environment can contribute to colony health and well being.
- How to use artificial diet-based bioassays for testing toxins and nutrients.
- The kinds of diet processing equipment, how they work, how they may fail.
- The handling of diet ingredients from procurement to use in diets (including proper storage and handling procedures).
- Learn by doing: most of the workshop principles will be illustrated with hands-on experiences.
- Group and individual activities will be directed at solving problems specific to participants laboratory/ rearing situations.

Workshop Cost for the week: $1,100

Contact us at: idri@insectdiets.com or (+) 1 520 577-6536 to find out more about the workshop or about the special hotel (1/2 block from U of A campus) rates for our workshop.

Publications

In Press

BAKRI, A.J. and J. HENDRICHS. Radiation Dose for Sterilization and Disinfestation of Tephritid Fruit Flies. In: Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa. (In press).

BAKRI, A, N. HEATHER, J. HENDRICHS and I. FERRIS. Fifty years of radiation biology in entomology: lessons learned from IDIDAS. Annals of the Entomol. Soc. Amer. (in press).

BILLINGSLEY, P.F., J.D CHARLWOOD, and B.G.J. KNOLS, Rapid assessment of malaria risk using entomological techniques: Taking an Epidemiological Snapshot. Kluwer Academic Publishers. Frontis series. (In press).

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. In: Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa. (In Press).

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. In: Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa. (In press).

CAYOL, J.P., W. ENKERLIN, A. BAKRI and J. HENDRICHS. Les mouches des fruits (Diptera, Tephritidae): ravageurs d'importance économique pour l'agrumiculture. In: 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. (In press).

DOWELL F.E., A.G. PARKER, M.Q. BENEDICT, A.S. ROBINSON, A.B. BROCE, R.A. WIRTZ. Sex separation of tsetse fly pupae using near-infrared spectroscopy. Submitted to Med Vet. Entomol. (In press).

ENKERLIN W.R. and M.M. QUINLAN. Development of an International Standard to Facilitate the Transboundary Shipment of Sterile Insects. In: Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa. (In press).

FILLINGER, U., G. SONYE, G.F KILLEEN, B.G.J. KNOLS, and N. BECKER. The practical importance of permanent and semi-permanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: Operational observations from a rural town in Western Kenya. Trop. Med. Int. Hlth, (In press).

KATSOYANNOS, B. I., N. T. PAPADOPOULOS, N. A. KOULOUSSIS and J. HENDRICHS. Effect of citrus peel substances on male Mediterranean fruit fly behaviour. In: Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance, May 6-10, 2002, Stellenbosch, South Africa. (In press).

MAREC, F., L.G. NEVEN, A. ROBINSON, M. VREYSEN, M. R. GOLDSMITH, J. NAGARAJU, G. FRANZ. Development of genetic sexing strains in *Lepidoptera*: From traditional to transgenic approaches. J. Econom. Entomol. (In press).

NDUNG'U, M., B. TORTO, A. HASSANALI, B.G.J. KNOLS, S. CHHABRA, and J. KERIKO. Larvicidal activity of extracts of five east African Meliaceae plants against *Anopheles gambiae*. Int. J. Trop. Insect Sci. (In press).

NIYAZI, N., C. CACERES, A. DELPRAT, V. WORNOAYPORN, E. RAMIREZ SANTOS, G. FRANZ and A.S. ROBINSON. Genetics and mating competitiveness of *Ceratitis capitata* (Diptera: Tephritidae) strains carrying the marker *Sergeant*, Sr^2. Ann. Entomol. Soc. Am. (In press).

ROBINSON, A. S., B. G. J. KNOLS, M. Q. BENEDICT, A. BOUSSAHA, G. VOIGT, P. ANDREO, Y. TOURE, J. HENDRICHS. Development of the Sterile Insect Technique for African malaria vec-

tors. I. Conceptual framework and rationale. Malaria Journal (in press).

SCHOLTE, E.J., B.G.J. KNOLS, R.A. SAMSON, and W. TAKKEN, Entomopathogenic fungi for mosquito control: A review. Journal of Insect Science. (In press).

SCHOLTE, E. J., B.G.J. KNOLS, and W. TAKKEN, Infection of the malaria mosquito *Anopheles gambiae* with the entomopathogenic fungus *Metarhizium anisopliae* reduces bloodfeeding and fecundity. J. Invert. Path, (In press).

SCHOLTE, E.J, B.G.J. KNOLS, and W. TAKKEN, Autodissemination of the entomopathogenic fungus *Metarhizium anisopliae* amongst adults of the malaria vector *Anopheles gambiae* s.s. Malaria Journal, (In press).

SUMBA, L.A., T.O. GUDA, A. L. DENG, A. HASSANALI, J. C. BEIER, and B.G.J. KNOLS, Selection of oviposition sites by the African malaria mosquito Anopheles gambiae (Diptera: Culicidae) is mediated by semiochemicals of microbial origin. Insect Science and Application. (In press).

SUMBA, L. A., K. OKOTH, A.L. DENG, J. GITHURE, B.G.J. KNOLS, J.C. BEIER, and A. HASSANALI. Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae* Giles (Diptera: Culicidae) on different types of aqueous substrates. J. Circadian Rhythms, (In press).

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 the 6th International Fruit Fly Symposium, May 6-10, 2002, Stellenbosch, South Africa. (In press).

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. (In press).

2004

ATKINSON, P.W., D.A. O'BROCHTA and A.S. ROBINSON. (2004) Insect transformation for use in control. In: Insect Pharmacology and Control - "Comprehensive Insect Biochemistry" edited by S.S. Gill, L.I. Gilbert and K. Iatrou, pp 234-254.

BAKRI A., HENDRICHS J., ENKERLIN W., CAYOL J.P., ROBINSON A., FERRIS I. (2004) The Tephritid Workers Database (www.tephritid.org); In: Proceedings of the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere, Ft. Lauderdale, Florida, May 16-21, 2004. USDA and University of Florida.

CACERES C. (2004)Current Status of Mass Rearing Technology for Olive Fly (*Bactrocera olea* Gmel.) (Diptera, Tephritidae). In: Proceedings of the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere, Ft. Lauderdale, Florida, May 16-21, 2004. USDA and University of Florida.

CACERES C. and WORNOAYPORN V. (2004) Relation Between Sperm Competition and Irradiation Dose in the Mediterranean Fruit Fly (*Ceratitis capitata* Wied.) (Diptera: Tephritidae). In: Proceedings of the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere, Ft. Lauderdale, Florida, May 16-21, 2004. USDA and University of Florida.

CACERES C., V. WORNOAYPORN, A. ISLAM, A. S. ROBINSON, M.T. VERA, H., DE LA VEGA, J. HENDRICHS and J-P. CAYOL. (2004) Mating incompatibility among populations of the South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae): implications for the Sterile Insect Technique. In: Proceedings of the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere, Ft. Lauderdale, Florida, May 16-21, 2004. USDA and University of Florida.

CHANG, C. L., C. CACERES and E.A. JANG A. novel liquid larval diet and its rearing system for Melon fly, *Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 97: 524-528.

DEPINAY, J.M.O., C.M. MBOGO, G.F. KILLEEN, B.G.J. KNOLS, J. BEIER, J. CARLSON, J. DUSHOFF, P. BILLINGSLEY, H. MWAMBI, J. GITHURE, A.M. TOURE, and F.E MCKENZIE. (2004). A simulation model of African Anopheles ecology and population dynamics for the analysis of malaria transmission. Malaria Journal, 3, 29 (21 pp).

FELDMANN, U. (2004). The sterile insect technique as a component of area-wide integrated pest management of tsetse, pp. 565-582. In I. Maudlin, P. H. Holmes and M. A. Miles (eds.), The trypanosomiases. CABI Publishing, Wallingford, U.K., 614 pp.

GBOLADE, A.A., N. LUKWA, D.T. KALENDA, C. BOËTE, and B.G.J. KNOLS, (2004). Guidelines for studies on plant-based vector control agents. In: Traditional Medicinal Plants and Malaria (M. Willcox et al., Eds.). CRC Press, FL, USA. pp. 389-408.

GOUAGNA L.C., B.A. OKECH, E. W. KABIRU, G. F. KILLEEN, P. OBARE, S. OMBONYA, J. C. BEIER, B.G.J. KNOLS, J. I. GITHURE and G. YAN (2004). Seasonality of Plasmodium falciparum infection and risk factors for gametocyte carriage in patients attending a rural health centre in Western Kenya. East African Medical Journal, 80, 627-634.

INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA). (2003). Biology, History, Threat, Surveillance and Control of the Cactus Moth, *Cactoblastis coctorum*. Vienna: IAEA.

IMPOINVIL, D.E., J. O. KONGERE, W.A. FOSTER, B. N. NJIRU, G. F. KILLEEN, J. I. GITHURE, J. C. BEIER, A. HASSANALI and B.G.J. KNOLS, Survival of the malaria mosquito *Anopheles gambiae* on plants from Mbita, western Kenya. Med. Vet. Entomol. 18: 108-115.

KILLEEN, G.F., A. SEYOUM and B.G.J. KNOLS, (2004) Rationalising historical successes of malaria control in Africa in terms of mosquito resource availability management. Am. J. Trop. Med. Hyg. 71:87-93.

KNOLS, B.G.J., L.A. SUMBA, T.O. GUDA, A. L. DENG, A. HASSANALI, J.C. BEIER, Selection of oviposition sites by the African malaria mosquito *Anopheles gambiae*. (Diptera: Culicidae) is mediated by semiochemicals of microbial origin. Int. J. Trop. Insect Sci. 24: 260-265.

KRASTEVA R., A. M. HANDLER, A. ZACHAROPOULOU, C. CACERES and G. FRANZ. 2004. Generation and initial analyses of transgenic medfly strains. In: Proceedings of the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere, Ft. Lauderdale, Florida, May 16-21, 2004. USDA and University of Florida.

MARQUEZ, J.G., M.J.B. VREYSEN, A.S. ROBINSON, S. BADO and E.S. KRAFSUR (2004) Mitochondrial diversity analysis of *Glossina palpalis gambiensis* from Mali and Senegal. Med. Vet. Entomol. 18: 288-295.

MATHENGE, E.M., G.O. OMWERI, L.W. IRUNGU, P.N. NDEGWA, E. WALCZAK, T.A. SMITH, G.F. KILLEEN, and B.G.J. KNOLS, (2004). Comparative field evaluation of the Mbita trap, Centers for Disease Control light trap and the human landing catch for sampling of malaria vectors in western Kenya. *American Journal of Tropical Medicine and Hygiene*, 70, 33-37.

MATTIOLI, R. C., U. FELDMANN, G. HENDRICKX, W. WINT, J. JANNIN AND J. SLINGENBERGH. (2004). Tsetse and Trypanosomiasis intervention policies supporting sustainable animal-agricultural development. Food, Agriculture & Environment 2 (2): 310-314.

MSHINDA, H., G.F. KILLEEN, W.R. MUKABANA, E.M. MATHENGE, L.E.G. MBOERA, and B.G.J. KNOLS, (2004). Development of genetically modified mosquitoes in Africa. Lancet Infectious Diseases, 4, 264-265.

MUKABANA, W.R., W. TAKKEN, G.F. KILLEEN, and B.G.J KNOLS,. (2004). Allomonal effect of breath contributes to differential attractiveness of humans to the African malaria vector *Anopheles gambiae. Malaria Journal*, **3**, 1. (8 pp.).

PETIT-MARTY N., M.T. VERA, C. CACERES and A. ROBINSON. (2004) Postzygotic isolation occurring between *Anastrepha fraterculus* populations from Peru and Argentina. In: Proceedings of the 5th Meeting of the Working Group on Fruit Flies of the Western Hemisphere, Ft. Lauderdale, Florida, May 16-21, 2004. USDA and University of Florida.

ROBINSON, A.S., G. FRANZ and P.W. ATKINSON (2004). Insect transgenesis and its potential role in agriculture and human health. Insect Biochem. and Mol. Biol. 34:113-120.

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 Programmes in the World. In: "Recent Trends on Sterile Insect Technique and Area-Wide Integrated Pest Man-

agement 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 Programmes. 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.

HENDRICHS, J. and A.S. ROBINSON (2003). Sterile Insect Technique. In: V.H. Resh and 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. 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.