Insect Pest Control Laboratory

Activities Report

2010
## Insect Pest Control Laboratory

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<th>Position</th>
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<tbody>
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**Insect Pest Control Laboratory**

**Summary 2010**

The Insect Pest Control Laboratory (IPCL) is an integral part of the Insect Pest Control Sub-programme and contributes to its global objectives to increase food security, reduce food losses and insecticide use, overcome constraints to sustainable rural development, and to facilitate international trade in agriculture commodities. The IPCL achieves these goals through the development and transfer of the sterile insect technique (SIT) package for key insect pests of crops, livestock and humans.

In the tsetse research group, R&D continued on the salivary gland hypertrophy virus that causes reduced fecundity in symptomatic *Glossina pallidipes* flies with consequently, stagnating or declining colonies. Work on the dynamics of the virus indicated that horizontal transmission was the main route of contamination in colonies maintained on the membrane *in vitro* feeding system. Promising results to manage the virus were obtained by using a strategy of clean feeding (each fly cage receives fresh blood) and to add the antiviral drug valacyclovir to the blood meals.

Upon request from the Government of Senegal, the IPCL initiated two new colonies of *Glossina palpalis gambiensis* in support of their effort to create a zone free of the fly in the Niayes area, north of Dakar. The impact of chilling and irradiation on late stage male pupae was studied which culminated in the development of a handling and transport protocol for these pupae. This protocol is currently validated in the field through weekly shipments of male pupae from Burkina Faso to Senegal.

The fruit fly genetic sexing group evaluated two Mediterranean fruit fly genetic sexing strains, which were constructed at the University of Göttingen using modern transgenesis techniques. Both transgenic strains produced in general fewer adults than the wild type (non-transgenic) strain but at the level of mass-rearing used, the strains appeared to be stable after 11 generations.

In the fruit fly mass-rearing and quality control group, various colonies belonging to the *Anastrepha fraterculus* and the *Bactrocera dorsalis* complexes were established. Field cage studies with *A. fraterculus* from Tucuman (Argentina), Vacaria and Pelotas (Brazil) indicated absence of mating barriers. Contrary, initial field cage studies between *B. dorsalis* and *B. carambolae* indicated a relatively high level of mating isolation supporting their current taxonomic status.

Significant progress was made with the further development of mass-rearing techniques for the olive fly *Bactrocera oleae*. More than 3 million eggs were collected over a period of 3 weeks using new production cages with a wax coated ovipositioning panel indicating that olive fly mass-rearing is becoming a reality.

Work continued in the mosquito research group on the development of suitable mass-rearing equipment and methods for *Anopheles arabiensis* and *Aedes albopictus*. A new larval tray design was successfully tested in a new rack system that can hold 50 trays. Promising results were obtained with a new industrial version of the larvae pupae separator. Initial data showed the feasibility of treating eggs (rather than larvae) of the genetic sexing strain of *An. arabiensis* to remove the female sex, and similar sterility levels were obtained with X rays as compared to gamma rays.
**Insect Pest Control Laboratory**

**Major Achievements 2010 in R&D**

*Tsetse Research Group*

**Colonies**

The Insect Pest Control Laboratory (IPCL) has been and continues to maintain colonies of tsetse flies depending on the needs and requests of FAO and IAEA Member States. Since several years, a colony of *Glossina pallidipes* (8000 producing female flies in 2010) has been maintained to provide biological material for research on the salivary gland hypertrophy virus (SGHV). The work is done in support of tsetse projects in Ethiopia and other Member States in Eastern Africa that aim at using the sterile insect technique (SIT) as part of their control efforts. In addition to the main *G. pallidipes* colony, two “clean feeding” colonies of this species were initiated in 2010 to assess the effect of this virus management technique (see below).

In 2009, a request was received from the Government of Senegal to carry out research in support of efforts to create a zone free of *Glossina palpalis gambiensis* in the Niayes area, north of Dakar. To be able to respond to this request, a colony of the target species was initiated with 8000 pupae received from the Centre International de Recherche-Developpement Sur l'Elevage en Zone Subhumide (CIRDES) in Burkina Faso (BKF). In 2010, the BKF colony was maintained at a level of 12 000 producing females to provide all the insects required for the experimental work. In addition to the BKF colony, a *G. p. gambiensis* colony was initiated with pupae from the target area in Senegal (SEN). Wild female flies were collected weekly from the Niayes in Senegal, transferred to an insectary in Dakar where the female flies were maintained for pupae production. Between October 2009 and September 2010, a total of 2185 pupae were shipped from Dakar to the IPCL. In December 2010, the SEN colony had reached a size of 450 females and had become self-sustaining. Initiation of a tsetse colony from wild flies is very challenging but due to close collaboration and intense interaction of IPCL staff with the counterparts in Senegal the SEN colony was successfully established.

**Glossina palpalis gambiensis**

The Government of Senegal opted not to develop its own mass-rearing capacity, and to procure the flies from the CIRDES in Burkina Faso where a colony of the target species has been maintained since the 1980’s. To enable shipments of male pupae from BKF to SEN, adequate handling and transport protocols needed to be developed. Female flies need to be retained in the colony and male and female *G. p. gambiensis* can only be separated at the adult stage. Adult sterile males are however too fragile to
be shipped such long distance. It was therefore hypothesized to exploit the difference in pupal period of male and female pupae followed by the chilling of the male pupae to delay emergence. Work was initiated to assess the effects of chilling (10, 12.5 and 15°C), irradiation dose and the combination of both on male pupae of different age. Parameters such as pupal development, emergence rate, male survival, insemination capacity and their mating performance in field cages were investigated. This research resulted in a handling and transport protocol that is currently being tested in Burkina Faso and Senegal.

Using sterile male insects in an SIT programme that originate from a different geographical area than those of the target area requires an assessment of their mating compatibility. It is essential for the success of a programme that mating barriers between the used and targeted strain are absent. Mating studies, undertaken in field cages that mimic closely the natural environment, revealed random mating between the SEN and BKF strains of *G. p. gambiensis*.

**Salivary gland hypertrophy virus (SGHV)**

Area-wide integrated pest management (AW-IPM) programmes that incorporate an SIT component require a thriving colony of the target species to provide sufficient numbers of males for sterilization and release. Development of a sizable *G. pallidipes* colony for the tsetse project in Ethiopia has been hampered by a high prevalence (up to 40%) of salivary gland hypertrophy (SGH) caused by the SGHV. Symptomatic flies have reduced fecundity causing colony stagnation or decline.

Work on the development of suitable virus management strategies was initiated several years ago and continued in 2010. The complete genome of the virus was sequenced which opened avenues for PCR and quantitative PCR’s techniques to study the dynamics of the virus. These studies revealed that both horizontal and vertical transmission occurs, but that horizontal (from fly to fly through feeding) transmission is the main mode in colony flies, whereas in nature vertical transmission (from mother to progeny) seems more important. Colony flies become infected with the virus through the ingestion of contaminated blood that they absorb using an *in vitro* membrane feeding system. A strategy of “clean feeding” was therefore tested, whereby the blood offered to the flies was only used once for each cage of flies. This strategy resulted in a significant reduction of the prevalence of SGH.

A second strategy that is being investigated is the use of antiviral drugs. Research continued to assess the impact of the two drugs acyclovir and valacyclovir on the prevalence of SGH and the virus. Feeding *G. pallidipes* colony flies for two years on blood mixed with the drug valacyclovir resulted in
acceptable productivity and reduced prevalence of SGH and SGHV load. Tests have been initiated to screen 15 other antiviral drugs.

Other strategies to manage the virus in the colony such as RNAi technology to silence the expression of certain genes, or neutralizing the virus infection with specific antibodies are being investigated, but results so far are not conclusive.

**Fruit Fly Genetic Sexing Research Group**

The last two decades, the IPCL has been the driving force behind the development of genetic sexing strains (GSS) for the Mediterranean fruit fly *Ceratitis capitata*. The development of these GSS allowed the removal of the female flies from the production line and the release of only male flies. This greatly increased the efficiency of the released males in the field and improved the cost effectiveness of the rearing, handling and releasing component of the SIT. The VIENNA 8 GSS that carries a white pupae (*wp*) and temperature sensitive lethal (*tsl*) mutation is now being used in all Mediterranean fruit fly facilities in the world.

The development of these GSS requires an inducible lethal factor and a linkage to the sex. Both classical genetics and modern transgenesis can be used to construct these strains. In recent years, the IPCL has been involved with screening and evaluating the performance of some transgenic strains (see activity report 2008). In 2010, two transgenic strains of the Mediterranean fruit fly, developed by the University of Göttingen, were evaluated. Both transgenic strains (which are not GSS strains but are being developed with the aim to induce sterility in a population without using radiation) produced in general 10% fewer adults than the wild type (non-transgenic) strain. At the level of mass-rearing used, the strains appeared to be stable (after 11 generations), although both strains seemed to suffer from the presence of the transgene.

In addition, two strains of the Mediterranean fruit fly being mass-reared in Guatemala and Hawaii were analysed with respect to their genetic status. It appeared that both strains contained a mixture of features that were only present in the VIENNA 7 and VIENNA 8 GSS. The construction of a new strain was initiated using the *wp* and *tsl* mutations of the VIENNA 8 during outcrosses with a Guatemala wild strain. Work was likewise continued with testing a GSS of *Bactrocera dorsalis* and with the construction of a new GSS strain of the melon fly *Bactrocera cucurbitae*.

**Fruit Fly Mass-rearing and Quality Control Research Group**

**Colonies**

Fruit flies are among the most severe pests of fruit commodities in the world and are classed in many countries as quarantine pests. Fruit flies have however, no quarantine status in Austria because of the cold winters, making the IPCL a unique place to culture, maintain and carry out research on these important pests.

The IPCL plays a central role in a new Coordinated Research Project entitled “Resolution of Cryptic Species Complexes of Tephritid Pests to Overcome Constraints to SIT Application and
International Trade”. The CRP was initiated in 2010 following increasing demands from Member States to resolve the uncertain taxonomic status of some fruit fly pests that exist as species complexes, i.e. fruit fly species that are morphologically similar and are not real separate species but only geographical variants. This is of crucial importance and results in possible unjustified trade barriers for important commercial fruit and vegetable commodities. In addition, some fruit fly populations that are grouped within the same species display different biological and genetic traits, which have important practical and economic implications for the effective use of the SIT. The IPCL has initiated the establishment of various new colonies of fruit fly species that belong to the *Anastrepha fraterculus* and *Bactrocera dorsalis* complexes, in addition to several other colonies. In December 2010, a total of 31 colonies of fruit flies were in culture at the IPCL, attracting researchers from a number of countries to carry out comparative studies on these major pest insects that are not possible in other parts of the world.

**Anastrepha fraterculus complex**

It is known that the South American fruit fly is composed of a complex of cryptic species comprising several morphotypes. The application of the SIT against this very important pest in South America poses problems when dealing with these reproductively isolated morphotypes that will not mate with adults of a different geographical region. Releasing sterile males of the wrong morphotype will not mate with the female flies of the target area and the SIT will not work. Earlier work at the IPCL already showed that mating barriers existed between populations from Peru and Argentina.

Colonies of *A. fraterculus* were initiated at the IPCL originating from Tucuman (Argentina), Vacaria and Pelotas (Brazil). During field cage studies, sexually mature adults from these three populations mated at random and yielded offspring that was fully fertile. These results, combined with earlier data seem to indicate that the area from Buenos Aires to Sao Paulo could be managed with one strain of *A. fraterculus*. Further work is planned with populations from Northern Brazil and Colombia.

**Bactrocera dorsalis complex**

Colonies were initiated at the IPCL of five species belonging to the *Bactrocera dorsalis* complex: *B. dorsalis*, *B. carambolae*, *B. philippinensis*, *B. invadens* and *B. papayae*. Initial field cage studies between *B. dorsalis* and *B. carambolae* indicated a relatively high level of mating isolation supporting their current taxonomic status. In contrast, mating studies between *B. papayae* and *B. dorsalis*, *B. papayae* and *B. philippinensis* and *B. dorsalis* and *B. philippinensis* revealed complete random mating.

**Olive fly rearing**

The olive fly *Bactrocera oleae* is the dominant pest of olives in the Mediterranean basin. It is highly invasive and has spread to California and Arizona in the USA and to Northern Mexico. For decades, farmers have been demanding alternatives to the use of insecticides that has been the traditional way of controlling this important pest. The development of the SIT for this pest was in the past hampered by difficulties with its rearing.

In 2010, significant progress was made with the further development of mass-rearing techniques for the olive fly. New production cages measuring 201 x 100 x 20.5 cm with a wax coated ovipositioning panel could be seeded with 1.2 litres of pupae. The maximum amount of eggs collected from...
such a cage was 91 ml or 3.1 million eggs over a period of 3 weeks. The trend in production efficiency continues to go upward indicating that olive fly mass-rearing is becoming a reality. The technology is being transferred to Israel, where a pilot field project is ongoing.

**Mosquitoes Research Group**

Since several years, various Member States have requested assistance with the development of the SIT package for disease transmitting mosquitoes. Initially, the research was focussed on the malaria transmitting mosquito *Anopheles arabiensis*, but expanded in 2010 to *Aedes albopictus*, an important invasive species that vectors diseases such as dengue and chikungunya. Mosquito SIT is at its infancy and therefore much of the research effort goes into developing rearing techniques that will enable the production of large numbers of the target insect. Except for the adults, mosquito eggs, larva and pupal stages are all water-based and the efficient handling of large volumes of water remains one of the great challenges in a mosquito mass-rearing facility.

Work continued with the further development of a flat larval holding tray that has a large surface area of shallow water that mimics natural breeding sites. A prototype rack system with a capacity to hold 50 larval trays was designed and the effects of reduced light conditions (the trays in the rack are only 3 cm apart) was assessed on development parameters. Water evaporation rates and water temperature in relation to the position of the tray in the rack were also assessed. As larvae do not develop synchronised, and in view of the short pupal period, the developed young pupae have to be removed from the production line each day. A stainless steel, industrial version of the larvae pupae separator was constructed and successfully tested. Finally, a new prototype adult holding cage was designed with two walls consisting entirely of netting. Ease of manipulation, egg oviposition, production rate etc. are currently being evaluated.

Mosquito-borne diseases are transmitted by female mosquitoes only, as blood is a requirement for egg development. Male mosquitoes do not bite humans and can therefore be sterilised and released without increasing disease transmission rates. However, such SIT programmes require the complete removal of the female sex to avoid introducing potential vectors into the target area. An *An. arabiensis* GSS strain that is based on an insecticide resistant mutation was developed a few years ago. Treating larvae of this strain with dieldrin, a potent insecticide, kills the female but not the male mosquitoes. In order to increase the rearing efficiency, work has been conducted on assessing the feasibility of treating eggs with dieldrin. The effects of egg age, dieldrin concentration and treatment time have been investigated.

The radiation dose administered to the male sex is one of the quality-reducing factors of released insects. The effect of gamma rays on egg hatch and survival of the male mosquitoes of the genetic sexing strain of *An. arabiensis* was therefore assessed for comparison with wild type strains. The level of sterility induced in *Ae. albopictus* after exposure to X rays was similar to results obtained with gamma rays from a $^{60}\text{Co}$ and $^{137}\text{Ce}$ source. In field cages, mating competitiveness of male *Ae. albopictus* seemed age-dependent with older males being more competitive than young males. Irradiation of pupae and treating eggs of the GSS strain with dieldrin reduced the number of sperm as compared to untreated GSS and wild type males. Whereas in untreated males the amount of sperm increased with age, the reverse was observed in irradiated males. This aspect is further being investigated.
CAPACITY BUILDING 2010

The IPCL hosted in 2010 four cost-free experts, i.e. Mark Schutze (Australia – 9 months – mating studies of Bactrocera dorsalis), Adalecio Kovaleski (Brazil – 8 months – mating studies with Anastrepha fraterculus), and Guy Hallman and Scott Myers (USA – 2 and 3 weeks respectively – post harvest cold treatment of fruit flies), two interns i.e. Odessa Madakacherry (USA – 7 months mosquitoes) and Arianna Pugglioli (Italy – 2.5 months - mosquitoes), and five fellows funded by the Department of Technical Cooperation, i.e. Nwe New Yin (Myanmar – 11 months – fruit flies), Henri Kariithi (Kenya – 2.5 months – tsetse), Mehrad Ahmad (Iran – 5 months – fruit flies and tsetse), Rania Ahmed (Sudan – 2 months – mosquitoes) and Giselle Ouedraogo (Burkina Faso – 2.5 months – tsetse). In addition, the IPCL hosted I. Kahn from Pakistan for 2.5 months in 2010 as a cost free fellow.

The IPCL hosted in addition five consultants i.e. Gratian Mutika (Zimbabwe - 12 months – tsetse field cage studies), Idrissa Kabore (Burkina Faso – 12 months – tsetse irradiation and cold treatment studies), David Damiens (France – 3 moths – mosquito sperm studies), Ihsan Ul Haq (Pakistan – 10.5 months – fruit fly behaviour studies) and Fabrizio Balestrino (Italy -2 months – mosquitoes mass rearing equipment development).

SERVICES PROVIDED 2010

The IPCL continuously receives requests from collaborators in Coordinated Research Projects, in Technical Cooperation projects, and from universities and research institutes for the supply of biological material. In 2010, the IPCL supplied 96,790 tsetse fly pupae (G. pallidipes, G. palpalis gambiensis and G. m. centralis) to five research institutes in Slovakia, South Africa, the UK, and the USA. In addition, the IPCL supplied 2400 pupae of the Bactrocera oleae olive fly and 0.05 ml of eggs, 3200 ml of VIENNA 8 (Ceratitis capitata) pupae, 400 ml of VIENNA 8 eggs, 400 pupae of Bactrocera cucurbitae melon fly, 13 600 Anastrepha fraterculus South American fruit fly pupae, and 500 Anastrepha ludens Mexican fruit fly pupae. In addition, the IPCL provided 11 kg of fruit fly diet. The fruit fly pupae were delivered to 14 research institutes in Argentina, Australia, Croatia, Czech Republic, Germany, Israel, Italy, Singapore, Spain, Mauritius, the Netherlands, and the UK.


