WORKING MATERIAL

Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control

*Fourth Research Co-ordination Meeting within the FAO/IAEA Coordinated Research Programme held in Kelowna, Canada, from 2 to 6 June 2014*

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**A. Situation analysis**

Pest species of Lepidoptera are among the most damaging species of food and fibre crops around the world. A recent analysis (ESA 2001) indicated that 19 of the 37 worst insect pest threats facing US agriculture are Lepidoptera (51%). Examples of globally important moth pests include codling moth *Cydia pomonella* (L.), diamondback moth *Plutella xylostella* (L.), oriental fruit moth *Grapholita molesta* (Busck), the American cotton bollworm or corn earworm *Helicoverpa zea* (Boddie), and pink bollworm *Pectinophora gossypiella* (Saunders). These pests cause severe losses to agricultural crops in vast areas all over the world, and are the target of very significant quantities of broad-spectrum and persistent insecticides in developed and developing countries. These interventions are unsustainable because the major economic, social and environmental consequences of these insecticide interventions are very large. The economic impact of these pests includes loss of yield, market access, and control costs.

The development of insecticide resistance in some Lepidoptera presents an additional threat to sustainability. Furthermore, many effective pesticides are being removed from the market due to increased recognition of long-term human health and environmental harm caused by their wide-spread use. Another consideration in favour of mounting area-wide interventions using SIT against moth pests is the increasing market pressure to reduce residues on food. The use of SIT becomes more attractive compared to other alternatives if costs can be decreased. Lastly, global increases in trade and travel have resulted in an increase in the rate of invasion of lepidopteran species, which threaten agricultural systems, markets, communities, and biodiversity on a worldwide basis.

**B. AW-IPM and SIT**

There is broad international consensus that intervention campaigns against such pests should be based on the area-wide concept of integrated pest management (AW-IPM), and that the sterile insect technique (SIT) can be considered as a key tactic for creating pest free areas or for pest management within IPM programmes. It has been known since the 1950’s that insect pests can be controlled or eradicated through a “birth control” method based on genetic manipulation, known as autocidal pest control, or the sterile insect technique (SIT). It involves the colonization and mass-rearing of the target pest species, sterilization of the insects through the use of gamma radiation, and their release into the field on a sustained basis and in sufficient numbers to achieve appropriate sterile to wild insect over-flooding ratios. The sterile insects need to find and mate with fertile, virgin insects, rendering the wild population infertile. Due to the absence of offspring, the natural pest population will decrease. The validity of this method has been demonstrated for many insect pests, including many moths, screwworms, tsetse, and fruit flies.

**C. SIT and Lepidoptera**

A refinement of the SIT for Lepidoptera, known as inherited sterility, involves lowering the irradiation dose moths receive (which would result in completely sterile female moths and partially sterile male moths with fully sterile offspring) and consequently increasing their competitive fitness. Moths treated with a lower sterilizing dose live longer, are stronger fliers, and mate more frequently than moths
treated with higher radiation doses. When inherited sterility systems are implemented into moth SIT programs, great improvements in programme efficiency can be obtained.

The use of SIT as a control tactic has many advantages, including species specificity and compatibility with the use of other area-wide control tactics such as mating disruption, biological control, cultural control methods and the use of bio-rational pesticides. It is an environmentally-friendly technology that can be used to address many of the world’s most difficult pest control problems.

There are many successful examples of SIT against Lepidoptera. These include operational containment, suppression and eradication programmes against the codling moth (Canada), pink bollworm (USA), cactus moth, Cactoblastis cactorum (USA), painted apple moth Teia anartoides (New Zealand), and false codling moth Thaumatotibia leucotreta (South Africa). In addition, there have been pilot projects to demonstrate feasibility in the field; e.g. gypsy moth Lymantra dispar (L.), tobacco budworm Heliothes virescens (F.), corn earworm, oriental fruit moth, carob moth Ectomyelois ceratoniae, and Asian corn borer Ostrinia furnacolis.

Further expansion of SIT to target other key lepidopteran pests will require improvements that increase the quality control and management of mass-rearing, irradiation, shipping, release and field assessment activities. In particular, key areas for further research and development are the factors and variables affecting the quality of insects and their field performance. In addition, new tools and methods to assess and predict the field performance of sterile insects are urgently needed. Finally, the enhancement of rearing systems through better understanding and management of genetic resources would contribute significantly to improving population suppression with SIT. Better systems for the selection and preservation of desirable traits within insect cultures would greatly enhance the efficacy and cost-efficiency of the released insects.

C.1. Factors and variables affecting field performance

Scope of the problem

Mass-rearing is essential to the sterile insect technique, but there are inherent quality problems that emerge when insect colonies are established and maintained for mass-rearing. The requirement for rearing large numbers of insects creates an artificial situation where production (quantity) is emphasized over the quality required for the competitiveness of the released insects. Genetic factors such as the founder effect, laboratory adaptation and genetic drift can be important, along with factors due to the stressful nature of the laboratory environment, such as larval crowding, insect pathogen load, and microbial contamination. Mass-rearing systems currently take little account of the differences between laboratory and field conditions. Insect handling and irradiation procedures can also have a major impact on insect quality.

These problems are manifested through a range of symptoms, but there is little understanding of the relative importance of these parameters on field performance. Sub-optimal competitiveness of mass-reared insects contributes to higher costs and lower efficiency at the programme level, since many more insects are needed for release and the programme duration is forced to be extended. These higher costs may cause loss of confidence in operational programmes, and limit the development of SIT against other potential targets. There are measures and systems used for quality control in mass-rearing, but more work is needed to identify and characterize the factors and variables that affect quality and field performance of released moths. There is currently little operational use of quality measures that reliably predict field performance, and little knowledge of how such measures could be used to improve mass-rearing procedures, enhance quality, and SIT outcomes.

Previous mass-rearing programmes focussed more on production volume than quality of insects. However, more recently it has been recognized that mass-reared insects used in SIT programmes need
to be of high quality, to ensure the efficacy and efficiency of the sterile insects when released in the field. The ability of mass-reared males to mate with wild females under field conditions has been recognized as vital to the SIT. Hence effective methods for monitoring and providing feedback on the quality and competitiveness of sterile insects are critical to the success of applying the SIT.

For the purposes of this CRP, a cross-cutting view has been selected that examines biological and operational factors affecting quality at all stages of the operation. Biological attributes that may affect quality include development rates, diapause, temperature adaptation, circadian rhythms, mating habits, flight capabilities, and many other characteristics. Operational attributes include cold storage, packaging and transport, release technology, and irradiation technique. Tests used to monitor the quality of laboratory reared insects must be reproducible, economical and simple.

Recent developments and future challenges

Much work has been done on the quality control of mass-reared tephritid fruit flies and some other insects. However, quality control in rearing moths has not been emphasized to the same extent, even though many workers have reported on various quality parameters. Even though most experience to date in mass-rearing moths has been in North America and Europe, in Australia and New Zealand, in the next few years, it is expected that this will change. For example, there has been a recent increase in interest in mass-rearing the codling moth in the temperate-climate countries of the southern hemisphere where apples and pears are grown commercially. In the case of the pink bollworm the programme has recently expanded from a containment programme in the San Joaquin Valley (700 000 acres) to an eradication programme covering 1.4 million acres in Texas, New Mexico, Arizona, Southern California, and Northern Mexico. This has involved an increase in production from 5 to 28 million moths per day.

Other recent research or operational programmes have targeted false codling moth, painted apple moth, carob moth, cactus moth, and diamondback moth. A new research programme targeting lightbrown apple moth _Epiphyas postvittana_ is also underway in Australia and New Zealand, which has drawn interest from California due to the recent discovery of an incursion there. These programmes all share the same need for a better understanding of the influence of biological and operational parameters affecting quality that can be measured and used to improve the field performance of released insects.

C.2. Tool and methods affecting field performance

Scope of the problem

There is a current lack of adequate tools and methods to link insect quality and field performance of sterile insects. Innovations in this area will provide the basis for feedback systems that deliver improved insect quality, efficacy and more effective population suppression. The assessment of field performance requires an adequate array of tools and methods for this purpose. The following examples illustrate areas with the need for improvements in this area. Laboratory, semi-field (e.g. field cage) and open field methods need to be integrated in order to improve our understanding of how to assess and improve insect quality from the factory to the field. Insects showing poor fitness in the field are likely to exhibit detectable characteristics in laboratory or semi-field assays, which are much less expensive than assessment at the point of open field release. Understanding the expression of moth fitness in a hierarchy of linked assays such as flight ability tests, wind tunnel flight performance tests, mating cage tests, field cage tests, and release-recapture tests would lead to the routine operational use of appropriate assays to manage quality.

Insect marking systems are essential to the process of tracking the fate of released insects, but can themselves affect quality. Incorporation of dyes into insect diets is a valuable approach, but may be
toxic or affect behaviour. External dyes, such as fluorescent powders, are useful, simple to apply and have a range of colours which is useful for evaluating different parameters. However, these external dyes can negatively impact on the response of males to pheromone sources. Rare earths are useful in principle, but very expensive to analyse. Other methods are therefore needed.

Current methods for determining moth population size and level of suppression due to SIT rely almost exclusively on pheromone traps, which only catch male moths. Consequently, there is no means of assessing half of the wild population or the levels of induced sterility in the native female population that result from the use of inherited sterility. In addition, lack of a sampling tool for female moths means that there is currently no way to assess the quality of female moths released in SIT programmes. Female moth trapping systems would therefore be highly desirable for several reasons.

A very considerable effort is routinely expended on checking traps for catches of wild moths and recaptures of released moths. Such information is often the only decision support tool available to programme managers, and forms the basis for major decisions on resource allocation. Often, effort is expended on checking empty traps, and major cost savings would result if this activity could be eliminated. In addition, the time and date of arrival of moths in traps cannot be determined from current routine visual inspection of traps, by field staff. This information would greatly enhance field programmes because changes in the overflooding ratio (sterile:wild) at the insect population level in near real time. Differences in pattern of trap catch could alert programme managers to subtle but important changes in moth quality, and lead to improved outcomes.

The release of sterile insects from ground and field equipment and aircraft is a key factor in the success of SIT, since the dispersion of sterile and wild moths should ideally be matched, and the release system should ideally not affect moth fitness. Some current release technologies have been shown to adversely impact on moth quality.

Recent developments and future challenges

Laboratory methods for assessing insect quality, such as flight ability, are routinely used in fruit fly SIT programmes, but their use has been limited to date in moth SIT programmes. Tethered flight mills have also been used to measure genetic variation in short and long range flight in this insect in the laboratory, and this trait was corroborated by field recapture studies. Wind tunnel flight ability has also been examined recently as a quality measure in the painted apple moth (Suckling et al. 2007). Field cage assessments have also been carried out with some success to examine mating competitiveness between sterile and untreated moths. The greater development and use of these approaches is urgently needed to permit the standardized assessment of moth quality, in order to improve the predictability of field performance.

Stable isotopes are a very promising alternative for marking insects, but further work is needed to develop this approach. Applications could include studies on mating competitiveness and dispersal, and the elimination of the risk presented by false positives, which can be very costly in operational programmes. The determination of the point of origin of trapped insects can provide programme managers with confidence that population movement is understood and that risks are being effectively managed.

Genetic markers in transgenic insects are a new approach to marking insects, although this approach may not be acceptable in all countries. Such markers can improve the identification of released insects and their mating status, enable the identification of the progeny of wild-sterile crosses, and verify progress with population suppression using the inherited sterility technique.

Female moth trapping systems would offer considerable benefits to operational SIT programmes by enabling the monitoring of female moth populations in the field, their mating status, quality, and release distribution. Attractants for some species of female moths have been known for some time, but
Recent developments of female lures for codling moth and other species are improving the prospects for this application in SIT.

New or improved electronic technologies could be deployed to increase the information obtained from trapped insects and lower the costs of trapping programme operation, by the integration of components currently available into insect trapping systems that could report to the web, electronic pagers or other media. Trap catch summaries could be reported and analysed in real time, if such catches were transmitted to a central data warehouse. Spatial analysis of geo-referenced trap data, together with other Geographic Information System (GIS) layers (e.g. crop damage and weather), would greatly enhance our understanding of insect population dynamics in the field. Furthermore, the identification of insect specimens through distance diagnosis would also avoid the need to check traps unnecessarily.

So called “smart traps” are being investigated for bio-security surveillance in several countries, including Australia, New Zealand and USA. The development and application of these systems for sterile insect programmes would lead to more cost effective and efficient field surveillance. Real time feedback on released insects could lead to rapid and adaptive changes to improve quality.

Other new information technologies could also lead to improved procedures that would improve insect quality assessment and outcomes. For example, developments in machine vision to record and analyse insect behaviour would offer the opportunity to quantify important quality factors such as flight ability, mating propensity and competitiveness. For example, preliminary analysis of the flight tracks of irradiated male painted apple moths in a wind tunnel showed differences from the behaviour of untreated controls in some individuals, although they arrived at calling females. This approach is likely to be amenable to automation, and could potentially be used in routine quality assurance.

C.3. Enhancement of rearing systems

Scope of the problem

The quality and cost of the mass-reared release insects is the single most important factor that limits expansion of existing programs and the initiation of new SIT programs. Much effort in rearing systems is invested in measuring quality control parameters but there is little relation between these laboratory measures and actual field performance. Insect colonies can exhibit the loss of desirable traits during the colony establishment phase and subsequently over time. The processes of selection under laboratory conditions are understood at a theoretical level, but have not been widely implemented in mass-rearing of moths. Furthermore, efforts have been made to understand the impact of factors such as diapause and fluctuating temperatures on moth quality. However, the quantification of such factors on the effectiveness of sterile insects to achieve population suppression has not been widely achieved. Improvements in this area would be of great benefit. This could involve developing new and improved methods for enhancing rearing systems, facilitating the selection for performance or fitness traits that improve colony establishment, refurbishment and production, as well as the field performance of released moths.

Recent developments and future challenges

Management methods have been developed for maintaining desirable traits during long-term culture of fruit flies and may apply for other insects. The use of a so called “filter system” has several advantages, since the parent colony is small and it is possible to keep the insects under low-density conditions. In addition, it is possible to apply sexual competition or directed selection in order to manage desirable traits, and if necessary, by establishing a new parent colony, the whole production can be easily switched to a new strain.
D. The Fourth Research Coordination Meeting

The 4th RCM of the RCP on “Increasing the efficiency of Lepidoptera SIT by enhanced quality control” was hosted by the Okanagan Kootenay Sterile Insect Release Programme, in Kelowna, British Columbia, Canada and held at the Coast Capri Hotel in Kelowna (2-6 June 2014). Sixteen contract and agreement holders from 13 countries attended the meeting. In addition, invited speakers from France (Dr Bernard Blum), Canada (Drs Luc Brodeur (Quebec), Cary Gates, Haley Catton, and Hugh Philip (BC)) gave presentations on the Regio Biocontrole (a new European initiative on area-wide approaches for codling moth control), the sterile onion maggot programme in Quebec, sterile leaf miner project, reduction in pesticide sales in the Okanagan valley, biological control of invasive weeds.

The last RCM of the CRP was very well organized and the great efforts of the local host Ms Cara Nelson are very much appreciated.

The first two days of the RCM were devoted to presentations of the participants. They presented the progress made with their research since the last RCM in Phoenix, USA as well as an overview of all research accomplished during the CRP.

The details of the progress made so far and the work plans for the last phase of the CRP can be found in this working material document.
E. Working Group 1: Factors and Variables that Affect Quality of Lepidopterans Used in SIT Programs

E.1. Biological attributes that may affect quality

Laboratory (mating and flight ability) bioassays and field (field cage and open field release) bioassays for the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), were conducted simultaneously to discern the ability of the different bioassays to predict quality and field performance of codling moths produced in a commercial mass-rearing facility. Moth quality was degraded by different levels of radiation during the sterilization procedure. Both the laboratory flight bioassay and the field cage bioassay successfully detected quality and performance differences that were relevant to moth performance in the field. However, the study data suggest that the field cage bioassay was a better predictor of the daily performance of males that had been released in the orchard than the laboratory flight bioassay. Conversely, data suggest that the controlled climatic conditions of the laboratory allowed the flight cylinder bioassay to be more sensitive in detecting daily fluctuations in the quality of moths caused by factors within the mass-rearing facility. Therefore, both laboratory and field bioassays may be required to provide feedback on quality and performance of mass-reared moths in a SIT program.

Characteristics were identified in *Lobesia botrana* male moths that may affect male reproductive success after being irradiated with 150 Gy. Compared to untreated insects irradiation (i) did not affect male or female emergence, (ii) did not affect male and female survival, (iii) did not affect male flying propensity to baited traps in a field cage. Male reproductive success was compared between untreated control males and irradiated males. The data showed that: (i) irradiated males and females mated significantly less (~5%) than untreated adults, and (ii) fertilization success of irradiated males and females was significantly reduced. The effect of irradiation on sperm characteristics showed (i) no significant effect on sperm production between males irradiated as pupae and untreated males, (ii) similar number of apyrene and eupyrene sperm were found in the spermatophore in the female *bursa copulatrix* after copulation. In summary, no abating effect was detected in 150 Gy irradiated male quality.

There was an effect of male size on spermatophore size. However, there appeared to be no effect of spermatophore size on reproductive success.

Circadian rhythmic release of *Spodoptera litura* sperm with respect to photophase and scotophase, was not affected by irradiation in sub-sterilized parent male moths and their F$_1$ generation males moths. Both in the photophase and scotophase, there was little quantitative reduction in sperm descent at the duplex level in P$_1$ (treated with 130 Gy) and in F$_1$ males (100, 130 Gy). The impact of irradiation on quantitative sperm descent was slightly more apparent in F$_1$ than in P$_1$ generation. The data indicated that the effect of irradiation was more apparent on eupyrene sperm descent than on apyrene sperm descent (although this trend was not statistically significant).

After standardization of an ‘in-vitro assay’ to assess sperm activation in adult *Spodoptera litura* moths, in-vitro sperm activation was studied in gamma irradiated males. The activity of apyrene sperm of irradiated male parents and their F$_1$ progeny was assessed with respect to (i) percentage sperm eliciting activity, and (ii) the degree (intensity) of activity in sperm. The impact of irradiation on percentage sperm activation of mated moths was apparent from 130 Gy onwards, but the sperm
intensity profile of mated male moths that were irradiated with 100-130 Gy was not significantly different from untreated moths (except 60-90 min after incubation). The proportion of sperm showing activity after mating was similar in irradiated and untreated $P_1$ moths with small differences between $F_1$ moths and $P_1$ moths. Mated males showed an increase in the percentage sperm activation and a decrease in intensity of sperm activity in $P_1$ moths (0 Gy, 100 Gy, 130 Gy). Overall, the degree of sperm activity was not influenced much unlike percentage sperm activation response that was relatively more affected at 130 Gy in both generations of this lepidopteran moth. These findings suggest that sperm dynamics can definitely be used as a one of the reliable tools to assess the quality of irradiated moths.

The effect of sublethal/substerilizing radiation was evaluated on the oxidative and nitrosative stress in *Spodoptera litura* and its antioxidant defence towards radiation stress. The present study was undertaken to evaluate the effect of gamma radiation on protein oxidation, lipid peroxidation, production of nitrite/nitrate metabolite and antioxidant defence levels which included non-enzymatic antioxidants (i.e.GSH/GSSG) and antioxidant enzymes activity (i.e. Catalase, SOD and APOx) in *Spodoptera litura* larvae gut. This study showed a close correlation between the radiation induced antioxidant defence system and the degree of radio-resistance in the lepidopteran insects, and this study would help us to understand the radio-resistant mechanisms operating in Lepidopteran insects in terms of antioxidants regime towards ionizing irradiation.

Age correlated irradiation influence on the phenoloxidase pathway, i.e the anti-oxidant defence mechanism in *Spodoptera litura* (Lepidoptera: Noctuidae) was assessed and its implication in radio-genetic ‘$F_1$ sterility’ and biorational pest suppression tactics described. Irradiation showed an inverse relationship with age with respect to the impact on developmental and transcriptional response in *Spodoptera litura*, indicating a decrease in radiosensitivity with increase in age. The extent of up-regulation of slppae-1, slppo-2, slsod and slcat transcript level in $F_1$ L6 developed from 100 Gy sub-sterilized male adult, was higher as compared to that in L6 survivors from L1 treated with 100 Gy. Evaluation of dual stress of radiation and microbial challenge indicated differential molecular response (transcript regulation) towards *E. coli* and *P. luminescens* in *S. litura*. The present study examined the influence of gamma irradiation on molecular responses related to phenoloxidase pathway and antioxidant defense operating in *Spodoptera litura* when treated in stages that have markedly differential radio-sensitivity. Radio-resistance was observed to increase with the age of the insect, and molecular responses (transcript upregulation) related to insect defence were less influenced when the moths were at a higher age. Differential transcript upregulation of biomolecules in response to irradiated and microbial challenge indicated the susceptibility of irradiated insects towards microbial agents. These findings might help optimize the gamma doses to be employed in inherited sterility technique for (pre harvest) pest suppression and (post harvest) phytosanitation and quarantine, and suggest compatible integration of biorational tactics including nuclear technology.

We also examined adaptive responses of antioxidant defence after high dose irradiation of intrinsically radio-resistant Sf9 insect cells. Sf9 cells were irradiated at doses ranging from 0.5 Gy to 200Gy. Lipid peroxidation and protein carbonylation levels were observed at 4h post-exposure along with glutathione (GSH/GSSG) profile as well as specific activities of redox active enzymes superoxide dismutase (SOD), catalase, ascorbate peroxidase (APOx) and glutathione reductase (GR). Human brain malignant glioma (BMG-1) cells were used for comparing radiation response of mammalian cells. Sf9 cells displayed significantly less radiation-induced ROS/RNS generation, protein carbonylation and growth inhibition as compared to mammalian cells. Sf9 cells have a higher basal APOx (~4 fold), catalase (~1.7 fold), SOD (~1.3 fold) activity and GSH level (~2.5 fold) compared to mammalian cells. A radiation dose-dependent increase in SOD, Catalase and APOx activity was found in Sf9 cells at least up to a dose of 100Gy, while maximum activity in mammalian cells was achieved by 10Gy. The present study suggests that insect cells carry a stronger
basal antioxidant system, which responds in tandem against ionizing radiation and could protect these cells against macromolecular damage, growth inhibition and cell death. Our study clearly demonstrated that the higher basic antioxidant status as well as radiation-induced increase in antioxidant enzyme activity in Sf9 cells might contribute to minimize the radiation-induced biomolecular damage. Since, the doses necessary to completely inhibit the recovery of proliferating in vitro population of these ovarian cells of Spodoptera frugiperda reasonably correlates with the doses needed in vivo to partially (100Gy) and completely (200Gy) sterilize adult Lepidopteran insects (Seth and Sehgal 1993, Seth and Sharma 2001), suggest that the Sf9 cells could be used as a model for optimization of inherited sterility technique (modified sterile insect technique -SIT) and molecular factors involved in its radio resistance could be modulated to reach desired sterilization with uncompromised mating efficiency for effective operation of sterile insect technique (SIT).

Spodoptera litura sperm transfer from irradiated male moths (P₁ & F₁) was ascertained at the spermatheca level where the sperm is retained, and used for fertilization with female gametes (eggs). The females mated to F₁ males received less sperm in the spermatheca as compared to those mated with P₁ males, although this effect was not drastic, and it could be correlated with successful matings (that transferred sperm up to spermatheca) and the degree of sterility. For example, the sperm transfer by P₁ and F₁ could be correlated with sterility being caused in F₁ generation (70-85%) in comparison to sterility in parent generation (50-55%).

Spodoptera litura male irradiation affected the mating success and remating propensity with female moths of different age groups. However, at each particular gamma dose, the age of the female had little effect on mating success, although the remating propensity of the females within each regimen was reduced with increasing female age. The impact of female age on its re-mating propensity with irradiated male moths was greater than that on its mating success. Further, the fertility of the females mated with F₁ males was similar among crosses when females were aged 0-1 and 2-3 days. There was a significant reduction in egg fertility in the crosses of 5-6 day old females mated withF₁ males derived from 100 and 130 Gy treated males.

The effect of mating status of female Spodoptera on the reproductive performance of irradiated male and F₁ male moth was assessed by using sequential matings. This species exhibited strong second male sperm precedence. The percent remating success of female was influenced by first mating, e.g. remating success would increase if paired with irradiated or F₁ male moths in first mating. Further, the percent egg viability was influenced by the second mating, e.g., the egg viability would decrease if paired with irradiated or  F₁ male moths in the second mating, that reflected sperm precedence.

Bioassays were carried out to compare quality of colony Conopomorpha sinensis reared on fresh fruit collected from the wild in terms of flight ability, longevity, mating performance. No significant differences between reared and the wild colony were observed. Studies on developing an artificial diet for Conopomorpha sinensis have been initiated and are ongoing.

Hypsipyla grandella (Lepidoptera: Pyralidae) (Zeller) is one of the most important forestry pests of Latin America and the Caribbean. It causes damage to cedar trees (Cedrela odorata L.) as the larvae feed on the meristematic tissue of new shoots, causing bifurcations in the plant and significantly reducing or annulling the commercial value of the tree. There is currently no effective and recommendable method for the control of H. grandella.

Female H. grandella were dissected and the spermatophore was identified. However, due to low sample sizes we were not able to statistically test differences in size of males, refractory period or mating frequency. As H. grandella had not been previously reared under controlled laboratory conditions, it was important to evaluate the mating behaviour of individuals reared using the new larval diet. Behavioural assays were carried out using three different sexual proportions (3:1; 2:2; and 1:3, male:female) but at the same density. Behaviours were categorized into: walking, wing fanning,
calling pose, copulation attempt and copula. Behaviours were comparable to previous reports in semi-wild conditions. Wing fanning was divided into long wing-fanning if adults fluttered their wings for more than 30 seconds, and short wing fanning if fluttering was observed in short bout of less than 10 seconds. Individuals were observed every 15 min for two consecutive nights from 9:00 am to 8:00 am under controlled laboratory conditions. Both males and females were active during the night, walking and wing fanning. Activity was observed at all sexual proportions. Half of all copulations were recorded in the 1:1 sex ratio. Copula duration was on average 1 hr 51 min, coinciding with the 2 hours previously reported for copulations in semi-wild conditions. This suggests that a 1:1 sex ratio may be ideal for rearing conditions. In addition, we measured the head width, thorax length and thorax width of all observed individuals. We found no significant difference in any of the size variables between males and females. There were no significant differences between the size of individuals that mated or failed to mate. Although this would need to be further evaluated in field cage tests, this suggests that in terms of quality control, the size of individuals may not be issue for copulation success. The effect of increasing larval density was tested on growth rate, pupal size and longevity. However, due to the cannibalistic behaviour of _H. grandella_, unacceptable levels of mortality were observed whenever more than one individual was reared in the same medium. Adult flight ability has not been tested due to low colony numbers.

A visit with Dr Jim Carpenter to Mozambique in July 2011 to assess rearing attempts for _Chilo sacchariphagus_ led to the development of a diet assessment protocol using established diets formulated for this insect from CIRAD in Reunion, MSIRI in Mauritius and a diet for _C. partellus_ from ICPE in Kenya. These diets were formulated and dispensed into multicell trays and sent to the rearing lab in Mozambique, and to the student at MSIRI, Mauritius. The first consignment of diet to Mozambique was lost in Mozamabican customs sheds, and the consignment to Mauritius had to be destroyed because of contamination with _E. saccharina_ neonates. The second consignments of diets reached their destinations, but no pupae could be obtained because of gross contamination of diets. The rearing of this insect was moved to controlled conditions in a quarantine laboratory in Stellenbosch, South Africa, and a diet formulation was chosen for complete development of larvae from eclosion to pupal development. In addition, mating and oviposition has been achieved under laboratory conditions using young maize plants in a gauze cage with wind passing through it (more details in CAPHANE, J. 2014 Mass rearing and radiation biology of _Chilo sacchariphagus_ Bojer (Lepidoptera: Crambidae. Transfer Report, University of Mauritius, Reduit, Mauritius.)

A diet incorporating rice mixed with fusarium as an alternative to chickpea is being investigated and is showing promise as an alternative, more productive diet to the current one. In addition, trials were conducted to mass rear _Eldana_ in large trays used for medfly SIT rearing, going through a series of trials using trays of increasing size. Four-liter plastic trays using the conventional diet resulted in significantly larger individuals, and a more “concentrated” development of larvae. In addition, the diet usage by larvae has been reduced from 8 to 5 ml. A shift to sago instead of corn cob grits as a dispersal medium for newly eclosed larvae on the surface of the diet resulted in significant decreases in diet contamination.

Tunisia is one of the most important tomato growers in the world. Tomato is the first vegetable crop in Tunisia in terms of area (26.000 Ha, 16%) and production (1.020.200 T, 33%). All parts of the plant in different developmental stages can be infested by _Tuta absoluta_ (leaves, stems and fruits green or red). There are four culture seasons each year with one greenhouse season (3% of area and 4% of production) and 3 cultures in the field (season, late season and autumn) covering 97% of the area and 96% of the production. _T. absoluta_ is a new and dangerous pest of tomato, reported in Tunisia since 2008. It causes 80-100% yield losses in greenhouse and open field. We found the life cycle is completed in 29 to 38 days depending on environmental conditions, especially temperature and humidity. Females that mated several times had a fecundity of 229±15 eggs/female and a fertility (egg
hatch) of 98.1%. Females which mated only once had a fecundity of 110±8 eggs/female and a fertility of 79.6%. *T. absoluta* is multivoltine, from October to May with 5-6 generations. There are 3-4 generations during March-July, and 3-4 generations August-November, resulting in 12-14 generations/year.

*Tuta absoluta* is one of the most important pests of tomato crops. It is native to South America but following its first detection in Spain in 2006, it spread into Europe, North of Africa and the Middle East at exceptional speed. The goal of this report is to show the studies carried out during the development of this project. The general objective was to establish the biological bases for the development of the sterile insect technique to control *T. absoluta* and its integration with biological control through the use of entomophagous insects in Argentina.

**Analysis of the effect of X-radiation:** An analysis was made on the effect of X rays on the emergence, fecundity and fertility of *T. absoluta* in order to find the dose at which females are sterile and males are partially sterile. The effect of radiation on adult emergence was evaluated exposing male and female pupae to increasing X-rays. Adult emergence decreased as doses of X-radiation increased, with the appearance of deformities such as malformed wings and bent legs at doses ≥ 36459.9 R (equivalent to 350 Gy). Besides, males and females obtained from irradiated pupae were mated with untreated counterparts to explore the effects of X-radiation on inherited sterility. (a) ♂ I x ♀ U cross (U: untreated, I: irradiated). Both fecundity and fertility of the untreated females were reduced by radiation, and the effect was stronger as the doses increased. Neither the longevity of parental males and F1 adults nor the sex ratios of the F1 and F2 generations were affected by X-radiation (F1 and F2: first and second generation of descendants of irradiated adults). Inherited sterility effects were manifested by a significant reduction in the F1 fecundity, F1 fertility, and the amount of larvae and pupae produced. Doses of 20834-26042.5 R (200–250 Gy) could be used to induce inherited sterility (IS) in *T. absoluta* males. (b) ♀ U x ♂ I cross. The minimum dose at which irradiated females were completely sterile was 20834 R (200 Gy). Besides, the larvae that descended from females irradiated with 15625.5 R (150 Gy) failed to reach pupae stage.

**Analysis of the impact of x-rays on cytological traits of Tuta absoluta.** We analysed the karyotype, the spermatozoa morphology and ratio of apyrene/eupyrene sperm in wild-type and X-ray irradiated individuals of *T. absoluta*. The diploid chromosomal number of *T. absoluta* was 2n = 58 including the pair of sex chromosomes, ZZ in males and WZ in females, which are the largest elements of the complement. Irradiation of pupae with an X-ray dose of 20834 R (200 Gy) generated different types of chromosomal rearrangements including translocations and fragmentations, resulting in altered chromosome numbers. The analysis of spermatozoa in *T. absoluta* revealed a notorious morphological difference between apyrene and eupyrene sperm bundles. Irradiation with doses of 10417-26042.5 R (equivalent to 100-250 Gy) did not have a significant effect on the apyrene:eupyrene sperm ratio. However, males irradiated with the dose equivalent to 31241 R (300 Gy) produced significantly more apyrene sperm than the non-irradiated males. All the doses applied influenced the morphology of eupyrene sperm bundles. The deformed eupyrene bundles could be used as bioindicators during the monitoring process after the release of irradiated males for IS programs. We found that these deformed eupyrene spermatozoa were transferred to the *bursa copulatrix* of the females. Males treated with 20834 (200 Gy) transferred a higher proportion of deformed eupyrene sperm than the untreated males. Our findings suggest that the detrimental effects of irradiation on sperm ratio and morphology, and on the karyotype, were responsible for IS in *T. absoluta*.

**Evaluation of the T. absoluta population suppression by irradiated individuals and their progeny in semicontrolled conditions.** We studied whether partially sterile *T. absoluta* males and fully sterile females are capable of suppressing wild populations of this moth in semicontrolled conditions. After irradiating *T. absoluta* pupae at 20834 R (200 Gy), males and females emerged were released at 10:1
(treated: untreated) overflooding ratio inside cages containing tomato plants. The number of eggs and larvae was recorded once a week during three months. The experiment was repeated with an overflooding ratio of 15:1. The use of an overflooding ratio 10:1 caused a decline of the larvae population compared to control cages but these differences were not statistically significant. When the overflooding ratio was increased to 15:1, the moth population in the cages with irradiated insects decreased significantly compared to control cages. On the basis of these results we can expect that with several releases of irradiated insects it is likely to reduce the wild population growth under greenhouse conditions.

Analysis of the compatibility of IS with biological control through the use of parasitoids and predators. We analysed the acceptability and suitability of *T. absoluta* eggs laid by irradiated parents to parasitism by the parasitoids *Trichogramma nerudai* and *Trichogramma pretiosum* under no choice and choice experiments. *T. absoluta* pupae were irradiated with 20834 R of X-radiation and the emerged adults were separated in pairs according to the following crosses: ♀ₚ × ♂ₚ (control), ♂₁ x ♀₁ (U: untreated, I: irradiated). Eggs (24-48 h old) obtained from the crosses were exposed for 24h to a single female parasitoid in a no choice experiment. All *T. absoluta* eggs were accepted for oviposition by *T. nerudai* and suitable for its development. However, eggs from irradiated females were significantly less parasitized than those from untreated females. *T. pretiosum* showed low parasitism on eggs from all crosses. In a choice experiment the following options were offered to a female wasp for 24h: 1) eggs from ♀ₚ x ♂ₚ vs. ♀₁ x ♂₁ crosses and 2) eggs from ♀₁ x ♀ₚ vs. ♀₁ x ♀₁. Both *T. nerudai* and *T. pretiosum* showed no differences in the parasitism of eggs from irradiated and non irradiated moths. These results provide evidence that sterile eggs were acceptable for oviposition and suitable for the development of these parasitoid species.

In addition, we studied in no choice and choice tests if eggs from parental crosses of *T. absoluta* were equally accepted for consumption by *Tupiocoris cucurbitaceus* (Hemiptera: Miridae) females. Parental crosses used were the same as above for *Trichogramma* experiments. In the no choice test, *T. cucurbitaceus* females consumed a high number of *T. absoluta* eggs regardless of their origin. In the choice test, *T. cucurbitaceus* females consumed as many eggs from untreated parents as eggs from parents with the irradiated male. However, the mirid females preyed significantly more eggs from irradiated females than eggs from untreated females. As irradiated females of *T. absoluta* have a low fecundity and their eggs are infertile, this preference would not be detrimental to the integration of the IS and the use of this natural enemy. In fact these eggs could be useful to keep the predator population in the system. On the other hand, the predator consumed a great and equal quantity of eggs laid by normal parents or females mated with irradiated males. In general, we can conclude that the integration of IS with the use of these natural enemies is a feasible strategy for controlling this moth pest.

Carob moth, *Ectomyelois ceratoniae*, is a major insect pest of dates, pomegranates, oranges and several other host plants in Tunisia. Larvae are polyphagous and attack both stored products and field crops in the Mediterranean basin and countries in the Near East region. This pest causes great economic losses and yearly infestation rates range from 20% in dates and oranges to 80% in pomegranates in Tunisia. Two experiments in Tunis (CNSTN and INAT) and Vienna (IAEA) were conducted to identify the sub-sterilizing dose for the carob moth. Five doses (100, 150, 200, 250 and 300 Gy) were tested. The parameters studied were fecundity, fertility, hatching eggs and adult emergence for 3 generations: parents, F₁ and F₂. The results showed no significant difference between the parameters studied in Tunis or those obtained at Vienna. Until 200 Gy, we still obtain a low emergence rate of adults for the F₁ offspring. For the F₂ offspring, we did not observe hatching eggs at 100, 150 and 200 Gy for Vienna experiment, and for Tunis experiment, we obtained a low rate of larvae development. As a conclusion of this work, 250 Gy is the radiation dose needed to sterilize females and sub-sterilize males of carob moth.
The competitiveness of sterile *Ectomyelois ceratoniae* males in laboratory cages shows that the ratio (5:1:1): 30♂ (non-irradiated)*30♀ (non-irradiated)*150 ♂ (irradiated) was a good ratio at 250 Gy to give an effective overflooding ratio between wild and irradiated males. In the field, the number of generations of the carob moth on pomegranates is about 3 to 4 generations. The competitiveness of sterile *E. ceratoniae* males were examined in a field trial. After irradiation, the moths were transported to the orchard and released. There were 3 successive releases spaced by one week. The number of moths released each week was 5000, 8000, and 7000, respectively. Irradiated males at 250 Gy preserved their ability to fly because they dispersed up to 150 meters from the point of the release. At the end of the trial, the % infestation was 2.8% in the control area and only 0.1% in the area treated with the release of sterile males.

E.2. Operational factors that may affect quality

Studies have been initiated on the orientation of F₁ male *Spodoptera litura* of sub-sterilized male parents towards pheromone traps (Fero-T™ procured from PCI) in field cages. The unmarked untreated moths and marked F₁ (100Gy) moths were released in the field cage, the total moths trapped were 69.4% which was almost similar to the control. From the total trapped insects, 52.9% moths were unmarked normal moths and 46.7% moths were marked F₁ (100Gy) or a ratio of 1:0.88 between unmarked N♂: marked F₁ (100Gy)-♂, which was a non-significant difference. When unmarked normal moths and marked F₁ (130Gy) moths were released, the total moths trapped was 63.3% which was slightly reduced as compared to the control. Present findings suggest that the attractiveness of F₁ males of sub-sterilized male parents (*S. litura*) that were treated with 100-130 Gy, towards pheromone traps in the field cages was almost similar to the control.

Studies were conducted on codling moth to examine factors related to insectary rearing and handling protocols by conducting laboratory bioassays of moth quality at several points along the production-release chain. Rearing and handling factors investigated included insectary moth collection system, insectary moth cold storage, irradiation procedure, packing protocols for moth distribution, transport of moths to the orchard, and transport and release of moth within the orchard. Degradation of moth quality was detected at many points throughout the moth collection-storage-irradiation-transport-release chain. The results from each trial were used as a feedback mechanism to make adjustments to protocols so that moth quality could be increased.

Following the successful eradication of *Cactoblastis cactorum* from Mexico and the elimination of *C. cactorum* from Alabama barrier islands, the USA/Mexico bi-national program established an additional mass-rearing insectary for the production of sterile moths. Laboratory and field bioassays were conducted on sterile moths from both insectaries (TIF and DPI). Bioassays and assessments included moth mass, moth longevity, percentage of female moths mated at time of collection from the insectary, percentage of female moths mated 24 h after collection, flight ability, percentage recaptured after release in the field, and mean distance captured from release site. Data from the quality assessments and comparisons between the 2 insectaries were used as feedback mechanisms to make protocol changes in both rearing and handling that improved sterile moth quality and performance. Quality and performance differences between moths from the two insectaries were detected by both laboratory and field bioassays. The results from trial 1 indicated significant loss of quality and performance by the DPI sterile moths, confirming the empirical field observations by workers in the Bi-national Cactus Moth Program. Parameters that indicated lower quality in DPI moths included longevity, percentage mated females at time of collection from the insectary, percentage females mating after collection, percentage of male moths flying out of the flight cylinders, percentage males recaptured following release in the field, and distance males were captured from release site. A careful examination of the differences between the two insectaries regarding the rearing and handling protocols provided guidance in selecting the most appropriate procedural changes that were made at
the DPI insectary. The scotophase was adjusted to insure that newly emerged adults remained in
darkness to reduce their activity and mating before they were chilled for collection and transport to the
irradiator. Adult activity was also reduced by lowering the temperature an additional 6 °C during the
adult collection period and 2 °C during the transport and irradiation phase. The third adjustment made
at the DPI insectary was to synchronize the photophase of the larval rearing period with the emergence
period. While the best way to understand how each procedural change might affect moth quality
would have been to conduct assessments after the implementation of each change, it was important to
improve the quality and performance of DPI moths expeditiously to maximize the success of the
ongoing Bi-national Cactus Moth Program. Nevertheless, relatively simple rearing and handling
protocol changes at DPI increased moth longevity, the ability of moths to mate following collection
from the insectary, the ability of male moths to fly from the flight cylinders, and the percentage of
male moths recaptured in the field after release. At the termination of this study, however, DPI moths
still demonstrated lower quality and performance than the TIF moths in the ability to mate after being
collected from the insectary, the ability to fly from the flight cylinders, and the distance captured from
the release site. Continuous quality monitoring and additional research are required to identify further
procedural changes that would enhance moth quality and performance.

One of the most important components of an SIT program is irradiation dose. Thus knowing the
organismal dose-response will enable selecting a dose that induces the highest level of sterility while
preserving the sexual competitiveness and quality of the sterile insect. Finding this balance in the
Lepidoptera is crucial because of the use of inherited (F₁) sterility, where the parent must be
competitive enough to mate while the offspring must be sterile. Manipulations of atmospheric oxygen
content have been shown to be an effective way of lowering post-irradiation somatic damage while
preserving sterility and improving sterile insect performance, particularly in fruit flies. We examined
the irradiation dose response of adults of the cactus moth, *Cactoblastis cactorum*, and the effects of
those doses on sterility, longevity, and F₁ performance, and whether a nitrogen conditioning-treatment
(anoxia) prior to and during irradiation affects these metrics. We found that male and female fecundity
was not impacted by dose or atmospheric treatment, but females were sterile at lower doses than
males. Eggs of irradiated parents took longer to hatch than those of unirradiated controls, and
offspring of moths irradiated in anoxia lived longer in the absence of food and water. Anoxia
conditioning rescued female fertility at intermediate doses but had no effect on male fertility, which
was always greater than female fertility at a given dose. Males generally lived longer than females and
anoxia had a strong effect in lowering the male mortality rate and extending lifespan at a given dose.
We show evidence that anoxia-conditioning prior to and during irradiation as part of a Lepidopteran
SIT program can improve parental and larval performance and longevity.

Exposure to ionizing radiation induces the formation of damaging free radicals in biological systems
that may reduce sterile male performance. Here, we tested whether exposure to an anoxic environment
for 1 h before and during irradiation improved male performance, while maintaining sterility in males
of the cactus moth, *Cactoblastis cactorum* (Berg). We showed that exposure to 1 h of anoxia increases
the moth’s antioxidant capacity and that irradiation in anoxia after 1 h of anoxic conditioning decreases
irradiation-induced oxidative damage to the moth's lipids and proteins. Anoxia treatment that reduced
oxidative damage after irradiation also produced moths with greater flight performance, mating
success, and longevity, while maintaining F₁ male sterility at acceptable levels for SIT. We conclude
that anoxia pretreatment followed by irradiation in anoxia is an efficient way to improve the quality of
irradiated moths and perhaps lower the number of moths needed for release SIT moth operations.

Trials were conducted to examine the effects of storage temperature on the recapture rate of the false
codling moth (FCM) in the SIT program in South Africa. In the first trial (2013-2014), irradiated (150
Gy) and non-irradiated male moths were held at 1-2 °C and 25 °C prior to release in the citrus
orchards. Data were collected on the number of males recaptured, the distance recaptured from the
release site, and the total distance recaptured from the release. In the second trial (2014), irradiated (150 Gy) male moths were held at 1-2 °C and 25 °C prior to release in the citrus orchards, and data were collected on the number of males recaptured, the distance recaptured from the release site, and the total distance recaptured from the release. In the first trial, the temperature at which the FCM males were held prior to release had a significant effect on the mean number of males recaptured and on the total distance from the release site where they were recaptured. Males held at 25 °C prior to release were recaptured at a higher rate and a greater total distance than males held at 1-2 °C. However, the radiation treatment did not significantly affect male moth performance. In the second trial, the temperature at which the FCM males were held prior to release had a significant effect on the mean number of males recaptured and the total distance males were captured from the release site. Males held at 25 °C prior to release were recaptured at a higher rate and a greater total distance than males held at 1-2 °C. The release date had a significant effect on the mean distance males were trapped from the release site, however, temperature did not significantly affect the mean distance males were captured from the release site.

Newly emerged adults of the European grapevine moth, *Lobesia botrana* (Denis and Schiffermuller) (Lepidoptera: Tortricidae), were irradiated with various doses of gamma radiation and crossed to unirradiated counterparts of the opposite sex. Fecundity was decreased when unirradiated females were mated with either 300- or 350-Gy-irradiated males. Adult males that were irradiated with 400 Gy and mated with unirradiated females retained a residual fertility of 2.7%. The radiation dose at which irradiated females were found to be 100% sterile when mated with unirradiated males was 150 Gy. The inherited effects in the F₁ progeny of irradiated male parents were examined at 100, 150, and 200 Gy. Fecundity and fertility of the F₁ progeny of males irradiated with 150 Gy and inbred or crossed with irradiated and unirradiated moths were also recorded. A significant reduction in fertility was observed when F₁ males mated with either F₁ or unirradiated females. According to sterility index, F₁ females who mated with F₁ males had greater sterility than when F₁ females were crossed to 150-Gy-irradiated males. Based upon the results of this study, 150 Gy of gamma radiation would be the optimal dose to use in a sterile insect technique and F₁ sterility program against *L. botrana*.

Even though a *Chilo sacchariphagus* laboratory colony has been established under quarantine conditions in Stellenbosch, South Africa, diet contamination caused colony reductions, as has an apparent “diapause”, causing really slow development of the larvae. This has delayed the radiation biology studies, and thus mating competitiveness studies.

Adult *Conopomorpha sinensis* emerging from the pupae irradiated at 0, 50, 100, 150, 200 and 250 Gy, were crossed with untreated adults in the following combinations: ①♂X♀; ②♂X♂; ③♂X♂; ④♂X♀.

The combination of - ♀(P) by ♀(P) in F₁ could be sterilized when parental pupae were irradiated at 200 Gy. Bioassays were carried out to compare quality of colony *Conopomorpha sinensis* reared on fresh fruit with collected from the wild in terms of flight ability, longevity, mating performance. No significant differences between reared and the wild colony were obtained.

A set of tethered flight mills connected to a microcomputer was used to assess flight distance, flight time, mean speed, and the highest speed of untreated male adults of Litchi stem-end borer. The flight distance, flight time, mean speed, and the highest speed of control male adults were 13926m, 29365s, 0.42m/s, and 1.01m/s, respectively in a 24h period. For every parameter, the untreated control moths scored the highest values among the three treatments (0,150 and 200 Gy), but the flight parameters between different treatments were not significantly different. It indicated that irradiation treatment with 150Gy and 200Gy had no significant effect on the flight ability of male litchi stem-end borer.

In a field trial, irradiation had no significant effect on the percentage of recapture and dispersal distance as compared with the untreated control. The median dispersal distance calculated by the
regression was 11.33m and 10.61m in the two experiments. The dispersal direction did not differ across the three treatments. The irradiation with 150 Gy and 200 Gy didn’t affect the litchi stem-end borer dispersal ability in the field. The value of the sexual competitiveness index of sub-sterile male adults by setting different mating ratios (the ratios of UF: TM: UM were 1:1:1, 3:1:1, 5:1:1 and 10:1:1) in laboratory field trials were between 0.49 and 0.62, which showed no significant difference. With a release ratio of TM: UF: UM was 5:1:1, the mean value of sexual competitiveness of the sub-sterile males in the field was 0.58, suggesting that both in laboratory or field, and the sub-sterile males successfully competed with wild litchi stem-end borers. Egg hatch of F1 generation in male adults from pupae irradiated at 200 Gy was 13.0%. No adult emerged in this treatment. The highest effects of irradiation were observed on F1 generation in all treated combinations in 3 generation. When the parental pupae were irradiated at 150 Gy, the combination -♂ (P) x ♀ (P) could last three generations, and all were effective.
F. Working Group 2: Tools and Methods to Assess Field Performance

The objective of this working group is to develop and improve tools and methods to assess, predict and enhance the field performance of released moths based on insect quality.

Scope of the problem

New tools and methods to assess and predict the field performance of sterile moths are urgently needed because there are currently limited or no reliable methods that can achieve this. New methods and technologies, such as stable isotope labelling, wind tunnel or semi-field scale assessment, will help to deliver a better understanding of the requirements for success that will enable the expansion of current programmes, target new insect pests, and increase the efficiency and effectiveness of pest control programmes.

This working group targeted the development of such tools and methods and aimed to deliver:

- One or more methods developed for laboratory assessment that predict the field performance of released moths based on insect quality;
- One or more methods identified for measuring the field performance of released moths at semi-field scale;
- One or more tools that enhance the measurement of field performance of released moths.

The following was achieved in 2009-2014 for each of the activities planned in the categories:

F.1. Theoretical Framework

A model for determining the overflooding ratio was developed for *Epiphyas postvittana* (LBAM) that was linked with other work (main findings of general interest: the minimum overflooding ratio for LBAM was estimated as 12:1); a limited effect from females in SIT was predicted. Important parameters that required estimation included:

- daily survival (or mean longevity) of adult males and females, wild and sterile
- relative performance of sterile males, in terms of finding mates
- relative performance of sterile females in terms of attracting males – are they any less attractive than wild ones?
- are females really sterile at a given dose, or do a few still produce viable eggs? If so, how fertile are they compared to wild females?
- mating competitiveness was assessed in field cages in Perth.

An MSc (Operations Research) modelling SIT in *Eldana saccharina* was submitted for examination (Potgieter L. 2012. A mathematical model for the control of *Eldana saccharina* Walker via the sterile insect technique. Department of Logistics, Stellenbosch University). Very high marks were given by both examiners, and the work was upgraded to a PhD, completed in 2014.

A first mathematical model was formulated for the population dynamics of an *Eldana saccharina* Walker infestation of sugarcane under the influence of partially sterile released insects. The model describes the population growth of and interaction between untreated and sterile *E. saccharina* moths in a temporally variable, but spatially homogeneous environment. The model consists of a deterministic system of difference equations subject to strictly positive initial data. The primary
objective of this model is to determine suitable parameters in terms of which the above population
growth and interaction may be quantified and according to which *E. saccharina* infestation levels and
the associated sugarcane damage may be measured. Although many models have been formulated in
the past describing the sterile insect technique, few of these models describe the technique for
lepidopteran species with more than one life stage and where F1-sterility is relevant. In addition, none
of these models consider the technique when fully sterile females and partially sterile males are being
released. The model formulated is also the first to describe the technique applied specifically to *E.
saccharina*, and to consider the economic viability of applying the technique to this species. Pertinent
decision support is provided to farm managers in terms of the best timing for releases, release ratios
and release frequencies.

A reaction–diffusion model was formulated for the population dynamics of an *Eldana saccharina*
Walker infestation of sugarcane under the influence of partially sterile released insects. The model
describes the population growth of and interaction between normal and sterile *E. saccharina* moths in
a temporally variable and **spatially heterogeneous environment**. It consists of a discretized reaction-
diffusion system with variable diffusion coefficients, subject to strictly positive initial data and zero-
flux Neumann boundary conditions on a bounded spatial domain. The primary objectives are to
establish a model which may be used within an area-wide integrated pest management programme for
*E. saccharina* in order to investigate the efficiency of different sterile moth release strategies without
having to conduct formal field experiments, and to present guidelines according to which release
ratios, release frequencies and spatial distributions of releases may be estimated which are expected to
lead to suppression of the pest. Although many reaction–diffusion models have been formulated in the
literature describing the sterile insect technique, few of these models describe the technique for
Lepidopteran species with more than one life stage and where F1-sterility is relevant. In addition, none
of these models consider the technique when fully sterile females and partially sterile males are
released. The model formulated here is also the first reaction–diffusion model formulated describing
*E. saccharina* growth and migration, and the sterile insect technique applied specifically to *E.
saccharina*.

A large scale operational field trial with *Epiphyas postvittana* (LBAM) was conducted in California,
and the data will enable estimates of the dispersal kernel (diffusion coefficient) for estimating release
rate and frequency, retention (daily survivorship - emigration).

**F.2. Trapping tools and studies**

Work continued on a female attractant for *Epiphyas postvittana* (LBAM) but results have proved
unreliable in this species so far. The results have been more promising for attracting female moths of
other leafroller species.

Work also continued on the development of a female attractant from host plant-derived kairomone for
improvement of trapping technology for assessing operational SIT programmes against diamondback
moth *Plutella xylostella* (DBM). There were a total of 12 bioactive peaks recorded by GC-EAD on the
cabbage headspace against the diamondback moth’s (DBM) antennae. GC-MS analysis was conducted
on cabbage headspace to determine the identity of these compounds and a tentative list was generated.
Preliminary field trials using some of these tentatively identified compounds, either singly or in
combinations caught some DBM, mostly males, but not in significant numbers. Further studies of
electrophysiologically-active compounds were completed along with a morphological study of
olfactory sensilla and characterization of olfactory receptor neurons response to host and non-host
odours.

Female DBM responses were assessed to potted intact healthy and larvae-infested cabbage plants in
no-choice wind tunnel bioassays. Mated females were significantly more attracted to the volatiles
emitted by larvae-infested than the intact healthy cabbage plants when presented separately in a wind tunnel. Total flight time taken for females to reach the upwind where the plant source was placed was significantly shorter for infested than intact healthy cabbage plants.

Host location, recognition and oviposition behaviour of mated DBM was assessed in no-choice and dual-choice observation cage bioassay. Mated DBM females were consistently more attracted to, and preferred to lay her eggs on the larval-infested cabbage over the healthy cabbage plants, both in no-choice and dual-choice bioassays. In all cases, females preferred to lay eggs on the leaves than any other parts of cabbage plants. Additionally, there was no significant difference in the total eggs laid between the beneath and upper leave surface within the same plant. Two behaviour, antennae rotation and ovipositor probing, were found to be important in detecting olfactory and gustatory cues during moth’s host search and host recognition before a host is finally accepted.

The effect of mating was assessed on female response to larvae-infested cabbage plants in no-choice wind tunnel bioassay. Wind tunnel results showed that mating did not affect the female response to cabbage host as the virgin and mated females showed similar percentage of response as well as flight duration to both intact healthy and infested cabbage host, when assayed separately.

Headspace of cabbage plants was sampled and samples processed with GC-EAD. Volatile emissions from both healthy and larvae-infested cabbage plants were sampled and tested on the excised antennae of DBM moths using a coupled gas chromatographic-electroantennogram (GC-EAD) detector. A total of 11 antennal-active peaks were detected from both infested/intact cabbage plants. There were some differences in the profiles of EAD-active compounds between male and female moths towards the cabbage extracts; three EAD-active compounds from infested cabbage were only active with females (no response from male antennae).

The effects of mating and conspecific larvae-herbivory was assessed on male and female diamondback moth response to cabbage host in flight competition. When mated and virgin moths were assayed together in a wind tunnel either to intact healthy or larvae-infested cabbage plants, there was no significant difference in the overall odour-modulated flight performance and total flight duration in both female and male sexes. However, there was a significant difference between sexes in the flight competition whereby females were more responsive than males to plant odour and took a significantly shorted time to reach to the plant source in upwind.

GC-MS analysis. We tentatively identified antennal-active peaks as dimethyltrisulfide, methyl dihydrothiophenone, hexyl acetate/hexenyl acetate, ethylidimethyl benzene/acetyltliophenone, 2-phenyl-2-propanol/ethyl heptanoate, 2-phenylethyl alcohol, 4-ethyl-6-hepten-3-one, menthone/tetramethyl benzene, 2-nonenal/octyl acetate, 2,6-nonadienal, geranyl acetone/carvyl acetate and caryophyllene alcohol/geranyl butyrate. However, the identity of these antennal active peaks was not able to be confirmed due to various reasons. Preliminary field trials, using some of the tentatively identified components, either singly or in combinations, were conducted to determine if these compounds are behaviourally active. Results showed that a few chemicals caught a low number of male and one female moths. Further work is need on the chemical identification and/or formulations but the approach looks promising.

A morphological analysis using a scanning electron microscope was carried out. The majority of antennal sensilla in female diamondback moth are trichoid sensilla, apart from coeloconica, basiconica and styloconica sensilla. At least three different types of trichoid sensilla were present according to the surface structure. Apart from the scale-like surface of non-porous trichoid sensilla, the presence of numerous pores on two types of the porous trichoid sensilla indicating their olfactory function.

During single cell recordings, overall, among 57 sensilla containing olfactory-receptor neurons (ORNs) with spontaneous firing activities, 42 sensilla contained ORNs responsive to some of the 39
compounds tested while 15 sensilla did not respond to any of the compounds tested. The 42 sensilla could be classified into Type A, B, C and D and appeared to contain 3 co-compartmentalized ORNs. Among 12 different ORNs in these sensilla 10 types of ORNs were responsive to some of the 39 compounds tested (i.e. two ORN types did not show any responses to the test compounds) thus exhibited narrow response spectra. Three types of ORNs (A1, A2 and A3) in Type A sensilla showed specialized responses to green leaf volatiles with the highest sensitivity to 1-hexanol and (Z)-3-hexenol with different response profiles, respectively. Two types of ORNs (B1 and B3) in Type B sensilla showed specialized responses to caryophyllene, (E)-β-farnesene and germacrene D, and with different sensitivities, respectively. Two types of ORNs (C1 and C3) in Type C sensilla showed specialized responses to (E)-β-farnesene, and each type of the ORNs showed slightly different sensitivities to this compound, respectively.

New pheromone blends of *Cactoblastis cactorum* with additional compounds were identified and evaluated in field tests. A commercial lure (used in mating disruption) for *Chilo sacchariphagus* was assessed for monitoring SIT.

The suitability of flight tubes to assess codling moth *Cydia pomonella* flight performance was investigated. Tubes of various diameters and heights were assessed under standard conditions in a laboratory. Results indicate that the number of moths placed in the tube and the diameter and height of the tube affect the flight ability of moths. The use of tubes of various dimensions has allowed for the determination of the optimum dimensions of tubes. The use of the tubes under various light regimes was assessed. Results indicate that the use of ultra violet light 1.5 meters above the base of the tubes was optimal. The method allows for assessment of flight ability after 6 hours. The method allows for the rapid assessment of moth flight ability under standard conditions.

With respect to the development of intelligent trapping systems, various options were investigated for *Cydia pomonella* and included smart traps using mini computers and camera cards. The costs associated with this were found to be relatively high. Literature searches revealed articles on the development and use of smart traps. More recently, GPS enabled “smart phones” have become available at relatively low cost. The technology is applicable in the field, but various aspects such as integrating the smart phone with wireless reporting to a central location have yet to be resolved.

A workshop on smart traps was organized in Phoenix and a discussion covered various aspects of the practical use of such traps, which varies between applications for surveillance, eradication and IPM. A prototype web-enabled camera trap was developed with a New Zealand web-based security company for fruit flies and moths. A prototype iPhone trap streaming to the web was also developed and demonstrated.

**F.3. Semi-field methods**

Field cages, transects and vineyard and urban grids were compared for key performance indicators of *Epiphyas postvittana* (LBAM) such as longevity, recapture rates, flight distance and mating rate. A ventilated outdoor horticultural tunnel was built and preliminary testing was undertaken in New Zealand.

Three series of replicated field cage competitiveness tests were carried out in Perth, Western Australia with LBAM. Tests were carried out in March 2008, April 2009 and September 2009. Techniques previously used to measure competitiveness of fruit flies were modified for use with moths. Field cages (2x3m) with an artificial *Ficus* tree placed in the middle of the cage were used. In one series of trials marked irradiated and unirradiated moths were released at dusk into the cages and mating pairs captured and mating type classified. The proportion of mating by sterile moths was used to determine the RSI competitiveness value. Pupae and moths irradiated at 200 Gy and 300 Gy had RSI values from 0.35-0.45. This indicates good competitiveness as a value of 0.5 indicates equal competitiveness.
between irradiated and unirradiated moths. In the second trial adult moths were irradiated with 250 and 300 Gy. Mating pairs were captured as in trial 1 but mated females were also placed individually into containers and egged. Percent egg hatch in combination with egg hatch from irradiated x wild moths and wild x wild moths was used to calculate the Fried C competitiveness value. Moths irradiated with 250 Gy had an RSI of 0.36 and C of 0.5 and with 300 Gy an RSI of 0.33 and C of 0.3. A C value of 1 indicates equal competitiveness between sterile and wild males and value between 0.2-0.4 are considered acceptable for sterile fruit flies. In the third series of trials moths were irradiated with 300 Gy and both C and RSI was measured but instead of marking and capturing mating pairs mating type was determined from egg hatch of individual females and spermatophore dissection. Additionally, male only was compared with bisexual release. Cages with release of irradiated male and females had RSI of 0.38 and C of 0.8 and with male only release a RSI of 0.64 and C of 2.2. The trials show that adapting fruit fly field cage competitiveness trials has good potential for use with moths. Moths irradiated with doses between 200 and 300 Gy showed good competitiveness as measured by the proportion of mating achieved by sterile moths. The ability to collect eggs and determine egg hatch from individual mated females has the potential to develop a non-intrusive and efficient way to determine the Fried C value. Good competitiveness was demonstrated using the C value with male only releases showing greater competitiveness. However, the field cage results showed wide variability and more trials are required to confirm these results.

An array of 64 traps was tested for recapture of *Cactoblastis cactorum* moths irradiated with different doses and released from a central release point in the field. Data were collected on recapture rate and distance from the release site.

An array of traps was tested in a ventilated outdoor horticultural tunnel for assessing thermal tolerance of *Cydia pomonella*. In addition, a protocol for the assessment of codling moth performance in the tunnel was developed. Results indicated that recapture rates of released moths was relatively low. Various changes were made within the tunnel; these included the addition of potted apple trees into the tunnel. It was concluded that the use of pheromone baited adhesive traps as an assessment method was not suitable as the trapping efficiency over relatively short periods was low. The use of ultra violet (UV) traps is being developed. The use of UV traps has a number of advantages and will attract both male and female moths.

Cage testing on overflooding ratios of *Epiphyas postvittana* (LBAM) was undertaken and data were collected to determine the effect of different overflooding ratios on population suppression.

A PhD thesis was examined and passed (Mudavanhu, P. 2012, Performance of sterilized *Eldana saccharina* Walker (Lepidoptera: Pyralidae) adults in mating and cage trials: Further steps towards its control using the Sterile Insect Technique. Department of Conservation Ecology and Entomology, Stellenbosch).

Main findings:

- Males exposed to 150 and 200 Gy called within an hour after darkness, wilds took 8 hrs
- Males exposed to 150 and 200 Gy mated with wild females 2 hrs before wild males did
- 150 Gy males remained in copula with wild for 2 hours, wild and 200 Gy for only 1.5 hrs
- On first night after emergence 150 and 200 Gy males mated with 3-6 wild females, while wild males mated with a max of 2; on second night, wild males mated with a max of 3 females, and the irradiated males a max of 2
- Under laboratory conditions, and without competition, males irradiated at 200 Gy (and 150 Gy) performed as well as, if not better than wild males at attracting and mating with wild females.
In general, despite differences in peak times of mating between the respective treatments, the data from both lab and field cages indicate that there is no evidence of any incompatibility between mass-reared, irradiated and wild *E. saccharina* strains. The results of the study thus provide the necessary evidence and confidence that the mass-reared *E. saccharina* strain currently produced at the SASRI insect rearing unit is suitable for use in SIT-based projects.

Pilot shade house trials were conducted to measure the impact of sustained releases of partially sterile adult males of the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) at an over-flooding moth ratio of 10T:1U (treated to untreated). Their efficacy in stopping *E. saccharina* incursions and suppressing wild populations was measured prior to testing under true season-long and area-wide conditions. Results from the current study demonstrated that releasing partially irradiated (200 Gy) adult male moths at the above-mentioned release rate significantly reduced sugarcane stalk damage, and also reduced the number of fertile progeny from F1 to succeeding generations in a stable *E. saccharina* population initiated in a cage-house. There were more damaged internodes per stalk in the control than in the sugarcane receiving regular releases of partially sterile male moths. Overall, there were significantly more undamaged stalks in the treated sugarcane than the untreated control. Furthermore, there were significantly more larvae per stalk retrieved from the control than from the treated sugarcane, suggesting that the sustained release of steriles was efficacious in reducing emergence of fertile larvae in the succeeding generations. The results of this study indicate that there is considerable scope for the Sterile Insect Technique (SIT) against this damaging pest of sugarcane.

Exposure of insects to gamma radiation in order to induce sterility, and mass-rearing to produce the large quantities required for the Sterile Insect Technique (SIT) are some of the many factors that may influence thermal tolerance at species and population level. The effect of mass-rearing and different radiation doses on the thermal tolerance of adult *Eldana saccharina* moths was investigated. Critical thermal maximum (CTmax) and critical thermal minimum (CTmin) were determined and compared for both genders of *E. saccharina* from five different treatments namely wild, mass-reared and three sterility dosages (150, 200 and 250Gy). Results showed that both wild and mass-reared moths had similar high temperature tolerances (CTmax: 44.3, 44.5°C respectively) and these values were significantly higher than those of moths exposed to gamma radiation (43.8, 43.7, 43.5°C respectively). However, wild moths had a significantly higher tolerance to low temperature (CTmin) than mass-reared and sterile moths (3.7, 4.4, 4.8, 4.9, 6.1°C respectively). In both CTmax and CTmin trials interaction between gender and treatment was highly significant (F (4, 90) = 4.19, p = 0.004, F (4, 90) = 3.71, p = 0.008 respectively). These results demonstrate the importance of knowledge of laboratory domestication and irradiation on thermal tolerance of *E. saccharina*. In addition to these data, further information on potential geographic and seasonal distribution will contribute to a better understanding of the working of SIT as an area-wide control strategy under different agro-ecological conditions.

A rearing protocol was developed for *Chilo sacchariphagus* together with Dr Jim Carpenter and sent to labs in Mozambique and Mauritius to provide insects for further research. A field visit was undertaken in Mozambique.

Codling moths were released in a large netted orchard in Elgin South Africa. A trapping grid consisting of twelve pheromone baited adhesive traps was established in the orchard. Moths were released at a central release point and traps were assessed up to 8 days post release. Results indicate that the efficiency of the traps in the netted orchard is significantly improved relative to traps in orchards. Codling moths radiated at 150 Gy and non irradiated moths were released in the netted orchard, results indicate that radiated moths were less competitive than non irradiated moths. Codling moths of various ages were also released in the netted orchard. Moths were held in Petri dishes at 5°C for 1, 2, 3, and 4 days. They were then released in the netted orchard and traps were assessed for the following eight days. Results indicated that age had an effect the number of male moths trapped.
Moths aged for four days were trapped up to seven days after release. The use of the netted orchard to assess moth quality was found to be suitable as high numbers of moths were recaptured over time and moths appeared to remain in the orchard. It was also observed that moths tended to disperse more evenly in the covered orchard when compared to releases in open orchards.

**F.4. Marking tools and studies**

Preliminary studies showed that natural abundance levels of isotopes can be used for marking insects and spermatophores of *Epiphyas postvittana* (New Zealand, Australia, USA), *Eldana saccharina* (South Africa) and *Cactoblastis cactorum* (USA), and diamondback moth (Malaysia) to determine mating success of sterile insects in the field, using tethered females.

The histological method for identifying F1 offspring of sterile males of *Cactoblastis* has been tested with insects mated in the field as part of an operational program.

Papers testing a histological method for identifying F1 sterile male *Teia anartoides* moths were published.

For SIT programs, to monitor levels of wild target pests and to assess the recapture rates of sterile insects, a method is required to distinguish between these two classes of captures on traps. Decisions in area-wide control programs using SIT are routinely based on captures of wild and sterile insects, but there are problems of misidentification with existing marking technology caused by loss or transfer of the markers such as the internal dye Calco Red or external markers with fluorescent powders.

Mating success of sterile insects and subsequent population suppression is the ultimate quality measure of operational performance in an SIT programme. There is currently a lack of adequate tools and methods to link insect quality to field performance of sterile insects. Due to the problems of trapping live female Lepidoptera, assessing sterile mating events using conventional egg hatch data is near impossible.

Stable isotopes offer a number of possibilities to study both the biology and ecology of moths and the implication of quality control in a mass rearing context. The advantages of stable isotopes pose no safety threat, and can be used in ecosystem studies in complex environments. Stable isotopes are non-radioactive and omnipresent in the environment, for example 13C with its one extra neutron constitutes one percent of all carbon atoms.

The isotopic signature of an organism is mainly dependent on what it eats; by feeding factory reared moths isotopically different diets to those of the wild population it is possible to intrinsically mark the moths. It is possible to use the natural abundant isotopic signatures of dietary products in particular sugar to intrinsically mark factory raised Lepidoptera. Lepidoptera fed on C4 cane sugar have a distinctly different isotopic signature to their wild C3 raised counterparts, however the extent to which this natal signature is retained unknown.

The majority of moth species feed on C3 plants in the wild which have a carbon isotope signature of around -28‰. However, almost all mass rearing facilities use cane sugar in the larval diet which is a C4 sugar source (with a signal of around -11‰) providing us with a simple method to differentiate released mass reared moths from wild moths. We tested whether we could use these differences for a wide variety of moth species.

Moth samples from the majority of the participating CRP Member States were analysed for isotopic signature as part of our participation in the CRP. This was used to determine whether natural isotopic signatures could be used as intrinsic markers to accurately and reliably define the origin of the moth, be it mass reared or wild. In addition, a new analysis technique for measuring of isotopic signatures based on laser technology was tested.
Research conducted during this CRP has demonstrated that natural differences in the isotopic signatures of moths reared on mass-rearing diets compared to those of wild moths reared on native species are excellent markers for establishing the source of individuals in a population. These isotopic methods provide us with a useful tool in improving the quality control of lepidopteran species as they offer opportunities to field test factory quality parameters without the confounding factors such as predation biases due to dye marking etc.

Studies in corn borer using the naturally occurring isotope signature differences between corn-C4 and C3 plants had been used to determine the paternal origin of the spermatophores. In addition successful isotope labelling experiments had been carried out in which male mosquitoes were highly labelled with $^{13}$C or $^{15}$N which allowed the transfer of labelled male sperm into female spermaphaceae traced. We set out to determine and develop a comparable natural abundance method.

During the CRP isotopic techniques were developed and tested to establish the paternal identity of wild female spermaphores, be they sterile mass reared male or non-sterile wild. These techniques have been successfully validated in the laboratory and have been successfully trialled in the field. These techniques really allowed us to answer the question, are mass reared specimens as competitive in the field as the wild types and are they competitive enough? This is a valuable tool as it really allows us to start linking production quality with field performance.

Another aim of the CRP was to test the laser-based technologies to measure isotope signatures in moths with a view to overcoming one of the adoption barriers to the uptake of isotope techniques in SIT programmes. The laser-based technology of Wavelength-Scanned Cavity Ring-Down Spectroscopy (WS-CRDS) system has substituted many of the current high-precision IRMS machines. Within the scope of this CRP this method of isotope analysis was investigated and proved to be equally a suitable method for insect analysis. The strategic advantages of the WS-CRDS systems are that they are compact, low-cost, robust and easy to use, making them ideal for use in field studies and in developing countries. Based on a daily 148 sample throughput and running the machine 48 weeks a year, the five year ameliorated capital cost per sample is around 0.36€, this is based on single insect sampling however if samples are three way pooled, which would still allow for accurate distinction, the cost comes down to around 12 cents per sample. It should be noted that the overall technology costs are relatively low as this is now an off the shelf technology which would require little method development. The research conducted as part of this CRP demonstrated that these machines could be used to determine SIT or wild paternal status based on isotopic analysis of spermatophores as it has been shown there is sufficient carbon in the spermatophore to be analysed using this machine.

**Projects initiated and funded as a direct result of the CRP**

USDA Funded: Development of Stable Isotopes and new Biochemical Tools for Identification of Sterile Insects and Determination of Pest Origin.

**F.5. Laboratory tools to assess fitness or movement**

Laboratory and field tests with LBAM were completed and two papers published.

A paper reporting results from arenas with controlled temperature was published.

Photo resolution on the thermal arena was not good enough to follow first instar *Eldana* larvae, and adults just did not move on the arena. Instead CT min and CT max comparisons were made between wild and lab reared adults, as well as adults exposed to 150, 200 and 250 Gy radiation doses. The wild moths had lower CT min and higher CT max values compared to the lab reared moths. As radiation dose increased the exposed moths became linearly less tolerant of colder and higher temperatures compared to the lab reared moths not exposed to radiation.
Exposure of insects to gamma radiation in order to induce sterility, and mass-rearing to produce the large quantities required for the Sterile Insect Technique (SIT) are some of the many factors that may influence thermal tolerance at species and population level. The effect of mass-rearing and different radiation doses on the thermal tolerance of adult Eldana saccharina moths was investigated. Critical thermal maximum (CTmax) and critical thermal minimum (CTmin) were determined and compared for both genders of E. saccharina from five different treatments namely wild, mass-reared and three sterility dosages (150, 200 and 250Gy). Results showed that both wild and mass-reared moths had similar high temperature tolerances (CTmax: 44.3, 44.5°C respectively) and these values were significantly higher than those of moths exposed to gamma radiation (43.8, 43.7, 43.5°C respectively). However, wild moths had a significantly higher tolerance to low temperature (CTmin) than mass-reared and sterile moths (3.7, 4.4, 4.8, 4.9, 6.1°C respectively). In both CTmax and CTmin trials interaction between gender and treatment was highly significant (F (4, 90) = 4.19, p = 0.004, F (4, 90) = 3.71, p = 0.008 respectively). These results demonstrate the importance of knowledge of laboratory domestication and irradiation on thermal tolerance of E. saccharina. In addition to these data, further information on potential geographic and seasonal distribution will contribute to a better understanding of the working of SIT as an area-wide control strategy under different agro-ecological conditions.

Understanding tolerance of thermal extremes by pest insects is essential for developing integrated management strategies, as tolerance traits can provide insights into constraints on activity and survival. A major question in thermal biology is whether thermal limits vary systematically with microclimate variation, or whether other biotic or abiotic factors can influence these limits in a predictable manner. Here, we report the results of experiments determining thermal limits to activity and survival at extreme temperatures in the stalk borer Eldana saccharina Walker (Lepidoptera: Pyralidae), collected from either Saccharum spp. hybrids (sugarcane) (Poaceae) or Cyperus papyrus L. (Cyperaceae) and then reared under standard conditions in the laboratory for 1–2 generations. Chill-coma temperature (CTmin), critical thermal maximum (CTmax), lower lethal temperatures (LLT), and freezing temperature between E. saccharina collected from the two host plants were compared. CTmin and CTmax of E. saccharina moths collected from sugarcane were significantly lower than those from C. papyrus (CTmin = 2.8 ± 0.4 vs. 3.9 ± 0.4°C; CTmax = 44.6 ± 0.1 vs.44.9 ± 0.2°C). By contrast, LLT of moths and freezing temperatures of pupae did not vary with host plant [LLT for 50% (LT50) of the moth population, when collected from sugarcane: - 3.2 ± 0.5 °C, from C. papyrus: - 3.9 ± 0.8°C]. Freezing temperatures of pupae collected from C. papyrus were -18.0 ± 1.0 °C and of those from sugarcane -17.5 ± 1.8 °C. The E. saccharina which experienced the lowest minimum temperature (in C. papyrus) did not have the lowest CTmin, although the highest estimate of CTmax was found in E. saccharina collected from C. papyrus and this was also the microsite which reported the highest maximum temperatures. These results therefore suggest that host plant may strongly mediate lower critical thermal limits, but not necessarily LLT or freezing temperatures. These results have significant implications for ongoing pest management and thermal biology of these and other insects.

A system for monitoring adult output from cyclone collection of LBAM was tested to improve the quality and production of insects.

Work was conducted to develop molecular tools that can be used to reveal gene flow patterns, genetic structures and dispersal distances of codling moth populations in China, and to assess whether the SIT can be used to control this newly introduced serious insect pest in China. Cydia pomonella is a serious invasive insect pest which is mainly in the northwest and northeast of China. This species has caused severe damage to the production of apple and pear in invaded areas. The sterile insect technique (SIT) is considered as a possible effective tactic to control C. pomonella in the invaded regions. The degree of gene flow among insect populations is important for the success of environment-friendly control technologies such as sterile insect release (SIT). Genetic variation of Cydia pomonella populations
from two geographically adjacent regions which are separated by stone deserts and huge mountains in a newly invaded corridor region of China was investigated using eight microsatellite loci. There was significant genetic differentiation between populations of the two regions. Populations from the same region showed similar genetic structure, but showed different genetic structure than populations from the other region. Compared with population pairs from different regions, population pairs in a same region showed higher $N_m$ and lower $F_{ST}$ values. Limited gene flow among populations from the two sampling regions was documented, which suggested that SIT can be implemented for area-wide control the pest in the special corridor of China.

To provide the population genetics of for area-wide control of the codling moth by SIT in China, eight microsatellite and three mitochondrial genes were used to investigate the genetic structure of the genetic diversity of the codling moth from the main distribution regions in north western (Xinjiang and Gansu Province) and north eastern (Heilongjiang Province) China. Both the *C. pomonella* populations from north eastern and north western China shared some haplotypes of the three genes with populations from Europe and other continents. Population from north eastern China showed different population genetics than populations from north western China, implying different invasion resource of *C. pomonella* in the separate north eastern and north western distribution regions. It is deducted that *C. pomonella* populations of north eastern China come from the far eastern region of Russia, whereas *C. pomonella* populations of north western came from the central Asian countries. The different population genetics background of *C. pomonella* populations in the north eastern and north western China should be considered before the application of the new control tactics like SIT.

The results indicated that SIT can be used as an area-wide control tactic to put down codling moth in China at the current early invasive stage in the country. The molecular markers applied in the study could be used to test of private alleles/haplotypes between factory SIT and wild populations in order to determine population introgression at the F1 generation.
G. Working Group 3: Quality Control Aspects in Relation to Rearing of Moths

The objective of this working group was to develop new and improved methods for enhancing rearing systems and to facilitate the selection of performance and fitness traits that improve colony establishment. During the period of the CRP, extensive progress was made with the development of mass-rearing and laboratory rearing methods that resulted in significant improvements to the quality of several species of moths reared in operational programs or in programs under development. These include: *Cactoblastis cactorum, Chilo sacchariphagus, Cydia pomonella, Eldana saccharina, Hypsipyla grandella, Lobesia botrana, Epiphyas postvittana, Pectinophora gossypiella, Thaumatotibia leucotreta, Tuta absoluta, and Ectomyelois ceratoniae.*

To prepare for implementation of possible SIT programs to target *Lobesia botrana,* several systems for rearing were developed to support research on radiation biology and other supporting technology. Three larval rearing diets have been developed. One of the systems (Israel) uses vertically oriented trays placed upside down at pupation so larvae fall to a paper substrate underneath allowing collection of clean pupae free of silk. This is a promising method which may allow the manipulation of pupae to use for efficient emergence systems and may also facilitate the development of reliable pupal irradiation methods for this species.

Major progress was made to develop a *Lobesia botrana* rearing system in Chile. In September 2012, testing of two artificial diets for rearing *Lobesia botrana* began in the laboratory. Diet A is an adapted artificial diet used for mass rearing of the pink bollworm of cotton, from USDA-APHIS Plant Protection Center and the USDA-ARS Western Cotton Research Laboratory mass-rearing facilities. The mix of vitamins and the mix of minerals incorporated in this diet were prepared in Chile, as these originals mixes were not available in the domestic market. The mix of minerals is a simile of Wesson salts and vitamins mix. Diet B is an artificial diet adapted from Thiery & Moreau (2005) and Stockel et al. (1989). A mix of minerals and vitamins was also added for diet B. Diet C was a modification of Torres-Vila et al. (2002). Thus, three diets were tested for rearing *Lobesia botrana.* Of the 3 diets the one that had the best results was the modified Torres-Vila diet. A vitamin mixture and salts were modified.

For each of the diets we evaluated female and male pupal weight and mortality, average fecundity and fertility per female. There were no statistical significant differences between pupal weights of the different diets. The lowest pupal mortality was observed in individuals from Diet B. For fecundity and fertility there was no statistical significant difference between Diets A and B. Fecundity and fertility was similar to that reported by Mondi & Corio-Costet (2000). Of the three diets, only diet B and C were sufficient to establish an artificial rearing of *Lobesia botrana.* It is necessary to conduct a cost analysis of production, as an important factor to improve mass-rearing. Only statistically significant differences were obtained in the mortality of female pupae, where diet A had the highest mortality.

A method for insect rearing was developed for *Lobesia botrana* consisting of 1 litre plastic cages adult cages. The eggs were collected from these plastic cages and placed standing vertically in the diet. These were placed in covered containers. Females lay eggs for 14 days and eggs are collected every 7 days (2 times). Temperature is kept constant at 23 +/- 1 C and 50% +/- 5% RH. The eggs are disinfected with bleach. On day 15 larval containers are opened so that pupae migrate to the paper. This rearing method is currently too expensive for up scaling to mass-rearing. Therefore, further research is needed to decrease costs, for example replacing imported products with local ones.
For *Cydia pomonella*, a new filler using *Pinus* sp. wood shavings and chips added to the mass rearing diet to comprise on average of 2.84% diet was tested. Moisture in the diet was reduced and no decrease in production or quality was observed and allowed a decrease in diet costs by 0.3%.

For *Conopomorpha sinensis*, optimal oviposition stocking ratios and optimal adult densities for maximum egg production were investigated. The stocking ratios were 1(male):2(female) and optimal adult densities is 30 female adults. Bioassays were carried out to compare the quality of a colony reared *C. sinensis* reared on fresh fruit with wild collected moths in terms of flight ability, longevity, and mating performance. No significant differences between reared and the wild colony were obtained. Evaluations of artificial diets have been initiated but so far rearing of *C. sinensis* has not been successful.

A diet development assessment protocol for *Chilo sacchariphagus* led to using established diets formulated for this insect from CIRAD in Reunion, MSIRI in Mauritius and a diet for *C. partellus* from ICIPE in Kenya. These diets were formulated and dispensed into multicell trays and sent to the rearing lab in Mozambique, and to the student at MSIRI, Mauritius. The first consignment of diet to Mozambique was lost in Mozambican customs sheds, and the consignment to Mauritius had to be destroyed because of contamination with *E. saccharina* neonates. The second consignments of diets reached their destinations, but no pupae could be obtained because of gross contamination of diets. A diet formulation was tested and showed there was complete development of larvae from eclosion to pupal development. In addition, mating and oviposition has been achieved under laboratory conditions using young maize plants in a gauze cage with wind passing through it.

For *Eldana saccharina*, a diet incorporating rice mixed with fusarium as an alternative to chickpea was developed and is showing promise as an alternative, more productive diet to the current one. Introducing four liter plastic trays, using the conventional diet has resulted in significantly larger individuals, and a more “concentrated” development of larvae. In addition, the diet usage by larvae has been reduced from 8 to 5 ml. A shift to sago instead of corn cob grits as a dispersal medium for newly eclosed larvae on the surface of the diet, has resulted in significant decreases in diet contamination. The fusarium diet has contamination problems, even though larval development in it is faster than in the conventional diet. Four liter trays, using the conventional diet has resulted in significantly larger individuals, and a more “concentrated” development of larvae. In addition, the diet usage by larvae has been reduced from 8 to 5 ml. A shift to sago instead of corn cob grits as a dispersal medium for newly eclosed larvae on the surface of the diet has resulted in significant decreases in diet contamination.

An artificial diet for the mass rearing of the carob moth, *Ectomyelois ceratoniae*, is already developed in the lab and it is composed by 60% wheat bran, 12% sucrose, 12% glycerin, 10% distilled water, 2.3% yeast, 2% salt mixture, 0.67% vitamin C, 0.67% Aureomycin, 0.13% methyl parabene et 0.3%. The development cycle of the insect is about 43.5 days in dates and 41.6 days on artificial diet at 25°C. More than 10 artificial diets were tested for *Tuta absoluta*. Examples of diets where we obtained no survival are as follows: 1st artificial diet: 60% wheat bran, 12% sucrose, 12% glycerin, 10% distilled water, 2.3% yeast, 2% salt mixture, 0.67% vitamin C, 0.67% Aureomycin, 0.13% methyl parabene et 0.3% lysine. No survival. 2nd artificial diet: water (150ml), Agar (3g), Maize flour (9g), Wheat germ (11g), Yeast (9g), Ascorbic acid (0,9g), Benzoic acid (0,3g), Grape seed oil (0,3ml), Nipagine (0,3g), Casein (3g). No survival. 3rd artificial diet: water (30ml), Maize flour (9g), Wheat germ (11g), Yeast (9g), Ascorbic acid (0,9g), Grape seed oil (0,3ml), Nipagine (0,3g), Casein (3g), Mixture sels (0,7g), Lysin (0,1g), Mixture sels (0,7g). No survival. Some progress was achieved with the following diet which yielded 30% survival: Water (1200ml), Agar (23g), bean (265g), Yeast (37,5g), Ascorbic acid (3,6g), Benzoic acid (1,8g), Formaldeide (3,6 ml), Nipagine (3g), Casein (30g).

A freeze dried diet preservation system was developed for *Pectinophora gossypiella* mass-rearing. Preliminary data show that the diet can be reconstituted and produces insects of similar quality to fresh
production diet.

While diet and breeding methods already exist for the Mahogany shoot borer *H. grandella*, these are expensive and inappropriate for production under controlled conditions. Specifically, modifications were made to the original larval diet ingredients, by substituting these with equivalents of lower price, and of greater abundance and availability. A new diet was developed substituting: 1) casein with powdered milk; 2) P-hydroxybenzoate with benzoate; 3) Wesson salts with regular veterinary salts; 4) Sorbic acid with Nipagin; 5) Cedar seeds with cedar leaves; 6) Chlortetracycline with Tetracycline. Cholesterol as an additional ingredient was removed from the new diet as it is naturally present in milk. Results showed that there were no significant differences between time spent as larvae, larval weight, time as pupae, fertility or fecundity of individuals reared on the new or original diet. The new artificial diet is more appropriate for potential mass-rearing in the future, less expensive than the original diet and uses ingredients that are more easily accessible. Also, we have eliminated the use of cedar seeds in the diet, which are seasonal and harder to obtain. Cedar leaves are available for longer periods and can be dried and kept until further use. This same diet can now also be used for neonate larvae.

We have also made modifications to the rearing method of eggs, neonate larvae, larvae, and adults. Specifically we have a new method for egg hatching, neonate larvae, new larval containers and new mating and oviposition cages. Using a wind fan directly placed in front of 30 x 30 x 30 mesh cages it is now possible to rear *H. grandella* in laboratory conditions. Oviposition was directed towards an artificial substrate utilizing larval frass as an attractant. This substrate could be further developed to act as an artificial oviposition device in the rearing cages. We have eliminated the use of fresh cedar leaves for egg hatch as now eggs are placed in a petri dish on a wet paper towel where they hatch. Neonate larvae are individually transferred to well plates with the new larval diet. Ball bearings or marbles are placed on top of each well plate to avoid larvae from escaping. Increased survival of neonate larvae with this new diet and method can be achieved compared to the traditional use of fresh cedar leaves. Third instar larvae are then transferred to individual petri dishes with diet. The use of petri dishes instead of jars increases survival of individuals and decreases space and diet use. Increasing larval densities have not been possible due to high mortality in multiply housed larvae, probably as a result of cannibalism. All these changes have permitted the maintenance of a colony within the laboratory for the first time for this species.

As part of the research into optimizing diet mediums and understanding the relationship between body size content and N or P, we assessed the stoichiometric variation of the Mahogany shoot borer. We found a negative correlation between dry weight and N or P body content. A mechanism involving high amounts of tRNA content associated to growth rate is frequently invoked for negative relationships between body weight and P. We suggest that although a fraction of the variance in stoichiometric composition of populations may reflect stoichiometric homeostatic efficiency or growth rate, a significant and so far frequently neglected fraction of this variance may be explained simply by the allometric relationship between body mass and tissue mass. A relatively higher content of lipids in heavier individuals could explain the observed relationship, as lipids are poor in N and P.

With respect to the development of adult collection systems, collection methods for *Cydia pomonella* were tested to improve collection efficiency. The reflection of UV from surfaces within the environment was found to reduce collection efficiency as moths were attracted to reflective surfaces. A solution to this problem was developed by reducing reflection and adding shielding. Testing of several wavelengths of LED UV lights were compared to standard fluorescent tube UV light and not found to enhance collection for *Cydia pomonella* however an LED UV light source was found to improve collection for *Epiphysis postvittana* over fluorescent UV bulb sources.
Adult collection systems using plenum and cyclone knock-down traps were tested for *Cydia pomonella* and *Eldana saccharina*. For *Cydia*, it was shown that the quality of moths collected from cyclone traps was better than those collected by a plenum based system. Key factors were the air speed and trap design. A knock-down temperature of 1 °C was shown to be optimal. An increase in collection efficiency was achieved which improved quality as demonstrated by increased flight ability, dispersal and longevity in the field.

For *Eldana*, a three emergence box adult collection system and plenum was constructed following the model of the system set used for *Thaumatotibia leucotreta* in South Africa in the XSIT program. A LED UV light system adapted from the light collection system used by the Phoenix Pink Bollworm facility was set in the adult collection tube at the entrance of the emergence boxes. Initial observations showed that *E. saccharina* moths in the laboratory moved upwards in the evening in the emergence rooms. As a result, the collection system was positioned above the emergence boxes. In collection trials, no moths were caught in the plenum. Investigations revealed that the moths did not move from the bottom of the emergence boxes. The system is currently being modified to bring the collection tubes to below the emergence boxes - much the same as at the XSIT system. Further trials will take place once these modifications have been completed.

An adult collection system for *Epiphysis postvittana* was developed with components from the *Cydia pomonella* rearing tray emergence method and the *Pectinophora gossypiella* cyclone knock-down trap system. The modular rearing system insect collection system includes new LED UV lights, high efficiency small scale dust collection systems, and enhancements to cyclone collection equipment. The development of a protocol for timing the adult collection helped to manage sex ratio from cyclone collections and improved production and quality of the egging cage system. The development of this new rearing system allowed a peak production of up to 500,000 *E. postvittana* per week which led to enough production to support an area-wide sterile insect release on a 2.6 km² pilot project in Long Beach, California to evaluate the potential for use of SIT to suppress LBAM populations in an urban infested area.

A study initiated in this CRP on the effects of thermal treatments of *Cydia pomonella* has been published (Chidawaniyka et al. 2010). Assessment of short term temperature treatments on codling moth pupae and adults has been inconclusive as treated moths were released in the field. The recent development and optimization of laboratory and semi field assessment methods will allow for further research. However, the successful field release of four day old codling moth adults which had been stored at 5°C is seen as a positive development.

The mass-rearing of the carob moth is needed between May and September when the wild insects are active in the field. To reduce the costs of the mass-rearing from October to April, we need to introduce the diapause in period of low production. We studied the effect of 3 factors that induce diapause in the field: photoperiod, temperature and the availability of food. Different cases were studied by combining temperature and photoperiod. Using the standard conditions of carob moth rearing (28°C, 16L:8D), we obtained 35% mortality, 90% adult emergence, a generation cycle of 36 days and a sex ratio of 1.

Changing the L:D periods, we obtained the following at 20°C:

16L:8D: 46% mortality, SR:1:1, 80 days to adult emergence
12L:12D: 54% mortality, SR: 59% of males; 150 days to adult emergence
0L:24D: 36% mortality, SR:1:1, 130 days to adult emergence
24L:0D: 100% mortality, no adult emergence
The most economic mode at 20°C seems to be a total darkness which maintain the population at latency state and give similar production on insects than the standard mode of mass-rearing.

If the eggs and L1,2,3&4 were kept at 28°C, 16L:8D and than the L4 instars were transferred at 10°C and 16L:8D, we obtain 59% mortality, 57% males and 47% of adult emergence in 143 days.

If eggs were kept at 28°C, 16L:8D and than transferred to 20°C, 12L:12D, two cases were studied.

24 hours age of eggs: only 33% of egg hatch and a high mortality of the young instars larvae.

72 hours age of eggs: 97% of egg hatch, 37% omortality for all the cycle and 64 days are needed by the carob moth to finish its development. The adults had good fecundity, fertility, longevity, SR but performance of the F1 generation was better and development was completed in 54 days.

When eggs and larvae (until L5 instars) were kept at 20°C, 12L:12D and than transferred at 14°C, 12L:12D, or if eggs only are developed at 20°C, 12L:12D and than transferred at 14°C, 12L:12D; we obtain for the two cases, respectively, 85 and 86% larval mortality and 14-15% nymphal mortality and no adult emergence.

**Conclusion of the proposed diapause protocol:** if we take reference of the diapause protocol established for the codling moth, the transfer of the carob moth from a temperature of 20°C, 12L:12D to a temperature of 14°C and a 12L:12D cycle gave us 100% mortality. So in this case we need more experiments since the codling moth is able to accept this conditions.

For evaluation of the effects of radiation hormesis, low doses of radiation given to *Spodoptera* larvae and cells were explored to determine if a radiation hormesis technique could be employed to increase quality in sterile moths. While conducting the radiobiological studies on *Spodoptera littoralis* (last instar larvae), and SF9 (*Spodoptera frugiperda* cell line), a stimulatory response was noticed in terms of increased SOD (Superoxide dismutase) and APOX (Ascorbate peroxidase) activity, and enhanced GSH (reduced Glutathione) at 0.5-1.0Gy. It is presumed that the extremely low ionizing dose range could be further explored for expressing radiation hormesis and rearing more viable and reproductively competitive moths to be employed in F1 sterility tactic.

For *Cydia pomonella*, work on thermal conditioning of the last larval instar (Chidawaniyka et al. 2010) increased quality of the moths in the field with increased dispersal ability under low and high temperatures.

For the False Codling Moth *Thaumatotibia leucotreta*, a new diet and packaging process are now been rolled out. It was also found that temperatures below 6 degrees Celsius had a detrimental effect on the flight ability of the males. Further studies into the cold tolerance of the moths and the effect of transportation will now be conducted. Amino acid content of current diet was investigated and other ingredients with similar nutritional profile were analysed for possible inclusion to develop a lower cost high quality FCM diet.

With the expansion of FCM SIT into the Sunday river valley (Eastern Cape Province, South Africa), transportation of moths needed to be critically analysed to ensure minimal quality degradation before releases. A new cardboard box system was developed that reduced the handling steps, allowed larger volumes to be handled during radiation, reduced condensation damage to moths and reduced cooling volumes required due to more efficient spatial use. Parameters relating to container capacity during radiation as well as cassettes directly connectable to aerial release devices will be investigated during 2012-2014.

A biomedical packaging system for organ transplant shipping was adapted from work in a previous CRP for shipping *Epiphyas postvittana* (LBAM) and *Cydia pomela*. The boxes kept cool stable
temperatures at 4-5 degrees C for up to 72 hrs. and allowed the shipping of over 600,000 high quality LBAM sterile moths to conduct a 3 month SIT pilot in California.

Egg sanitation treatments were evaluated for pathogen control in rearing systems. For *Epiphyas postvittana*, a protocol for treatment of eggs was developed using chlorohexadine glutamate (CHG) which resulted in improved eclosion in rearing trays and reduced pathogen incidence. CHG is a low toxicity alternative to eggs treatment with formaldehyde and other harsh sterilants but was infective at reducing mild incidence when mold contamination levels are high because mold spores are only partially killed. Because of this CHG is not a recommended sterilant. An ozone treatment for *Cydia pomonella* was tested and found to be ineffective for sterilization of diet trays and laboratory rearing areas as well as egg sheets.
G.1. CRP Outputs List

Peer reviewed publications


(Epiphyas postvittana) using a four-component sex pheromone blend. Crop Protection 42: 327-333.


**Book chapters**


**Conferences**

1. Cagnotti, C. L., M.M. Viscarret, E. Botto and S.N. López. 2010 Exploratory studies of radiobiology with X rays on Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) with a view to develop the Sterile Insect Technique”. XXXIII Argentinian Congress of Horticulture (Rosario, Santa Fe, Argentina; 2010).


3. Cagnotti, C. L., Viscarret, M.M., Botto, E. and S.N. López. 2012 Studies of radiobiology with X rays on Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) with a view to develop the Sterile Insect Technique VIII Argentinian Congress of Entomology (Bariloche, Río Negro, Argentina; 2012).


27. **Lauría J.P., Carabajal Paladino L., Cagnotti C., Muntaabski I., Cladera J.L. and S.N. López. 2012.** Characterization of the euspermatozoa transferred by irradiated *Tuta absoluta* males (Lepidoptera). XV Latinamerican Congress of Genetics, XLI Argentinian Congress of Genetics, XLV Congress of the Genetic Society of Chile and II SAG-Litoral Regional Meeting (Rosario, Santa Fe, Argentina; 2012).


29. **Li XY, and Chen MH. 2013.** DNA barcoding of four Tortricidae species (Lepidoptera: Tortricoida: Tortricidae) from China .5th International Barcode of Life Conference, October 27-31, 2013, Kunming, China.


BSc Thesis


Lauría, J.P. Characterization of the euspermatozoa transferred by irradiated Tuta absoluta males (Lepidoptera) and of the impact of X radiation on cytological characteristics of the F1 generation”. Facultad de Ciencias Exactas, Químicas y Naturales, Universidad de Morón. In preparation. Advisers: L. Carabajal and S.N. López.

MSc Thesis


PhD Thesis


Cagnotti, C. Development of the sterile insect technique to control of the tomato moth Tuta absoluta (Lepidoptera: Gelechiidae)”. Facultad de Ciencias exactas y Naturales, Universidad Nacional de Buenos Aires. In preparation. Adviser: Eduardo Botto.


Projects initiated and funded as a direct result of the CRP

USDA Funded: Development of Stable Isotopes and new Biochemical Tools for Identification of Sterile Insects and Determination of Pest Origin.
<table>
<thead>
<tr>
<th>Narrative Summary</th>
<th>Objective Indicators</th>
<th>Verifiable</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Objective:</td>
<td></td>
<td></td>
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</tbody>
</table>
| To provide the basis for improved efficacy of lepidopteran (moth pest) SIT through the development of systems that enable assessment and prediction of the field performance of sterile insects | N/A | N/A | | • The problems caused by pest moths will remain of regional / interregional relevance  
• SIT will remain an important component of area-wide integrated pest management campaigns against moth pests. |
| Specific Objectives: |                      |            |                       |                      |
| To identify and characterise factors and variables that affect quality and field performance of released moths. | N/A | N/A | | |
| To develop and improve tools and methods to assess, predict and enhance the field performance of released moths based on insect quality. | N/A | N/A | | |
| To develop new and improved methods for enhancing rearing systems, to facilitate the selection for | N/A | N/A | | |
### Narrative Summary

Performance or fitness traits that improve colony establishment, refurbishment and production, as well as the field performance of released moths.

### Objective Indicators

**Expected Outputs (1)**

Factors and variables that affect moth quality correlated with field performance

<table>
<thead>
<tr>
<th>Objective Indicators</th>
<th>Verifiable Indicators</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors and variables that affect moth quality correlated with field performance</td>
<td>One or more biological attributes and operational factors identified and characterised.</td>
<td>Biological and operational factors reported in oral or written form.</td>
<td>Appropriate biological attributes and operational factors exist and can be measured.</td>
</tr>
</tbody>
</table>

### Means of Verification

#### Quality Assessment

- Quality assessment protocols used by researchers and practitioners in ongoing projects.

#### Important Assumptions

- Methods investigating fitness traits have been reported in oral or written form.

### Important Assumptions

#### Assumptions

- Enhanced rearing systems are agreed and accepted by operational programme managers.

### Means of Verification

#### Accessible Tools and Methods

- New and improved methods and tools are agreed and accepted by operational programme managers.

### Narrative Summary

Enhanced rearing systems that enable assessment and prediction of the field performance of sterile insects

<table>
<thead>
<tr>
<th>Narrative Summary</th>
<th>Objective Indicators</th>
<th>Verifiable Indicators</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced rearing systems that enable assessment and prediction of the field performance of sterile insects</td>
<td>One or more methods evaluated that enhance rearing systems based on fitness traits, colony establishment, refurbishment and production</td>
<td>Methods enhancing rearing systems have been reported in oral or written form.</td>
<td>Enhanced rearing systems are agreed and accepted by operational programme managers.</td>
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</tbody>
</table>

### Important Assumptions

#### Important Assumptions

- Methods investigating fitness traits have been reported in oral or written form.

### Objective Indicators

**Expected Outputs (2)**

New and improved tools and methods to measure moth quality

<table>
<thead>
<tr>
<th>Objective Indicators</th>
<th>Verifiable Indicators</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
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</thead>
<tbody>
<tr>
<td>New and improved tools and methods to measure moth quality</td>
<td>One or more methods developed for laboratory, semi-field and field assessment that predict the field performance of released moths based on insect quality</td>
<td>Accessible tools and methods for laboratory, semi-field and field assessment.</td>
<td>New and improved methods and tools are agreed and accepted by operational programme managers.</td>
</tr>
</tbody>
</table>

### Means of Verification

#### Quality Assessment

- Quality assessment protocols used by researchers and practitioners in ongoing projects.

#### Important Assumptions

- Methods investigating fitness traits have been reported in oral or written form.

### Important Assumptions

#### Important Assumptions

- Enhanced rearing systems are agreed and accepted by operational programme managers.
<table>
<thead>
<tr>
<th>Expected Outputs (General)</th>
<th>Objective Indicators</th>
<th>Verifiable</th>
<th>Means of Verification</th>
<th>Important Assumptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Publications in peer-reviewed journals and final results published in a special volume</td>
<td>Papers drafted and submitted</td>
<td>Journal with published papers</td>
<td>Data is adequate for publication</td>
<td></td>
</tr>
<tr>
<td>New and improved general and specific quality measures for moth SIT programmes</td>
<td>Recommendations available</td>
<td>Progress report</td>
<td>Recommendations can be agreed upon by operational programme managers</td>
<td></td>
</tr>
<tr>
<td>New or refined methods and protocols for improving moth quality in operational SIT programmes</td>
<td>Methods and protocols available</td>
<td>Progress report</td>
<td>Methods and protocols can be agreed upon by operational programme managers</td>
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Final FAO/IAEA Research Co-ordination Meeting on
‘Increasing the Efficiency of Lepidoptera SIT by Enhanced Quality Control’

2-6 June 2014
Coast Capri Hotel, Kelowna
Canada

Monday 2 June 2014

OPENING SESSION

08.00 – 08.10  Steve Thomson, Minister of Forests, Land and Natural Resource Operations, BC - Official opening of the RCM.

08.10 – 08.20  Welcome address by Duane Ophus, SIR Board Chair

08.20 – 08.30  Welcome address by Fred Steele, BC Fruit Growers President

08.30 – 08.40  Cara Nelson / Marc Vreysen - Administrative details, objectives of the meeting and outline of the agenda.

SESSION 1:  Chairperson: Suk Ling Wee

08.40 – 09.00  Key note address: Cara Nelson – Twenty years of successful implementation of the SIT against codling month in Canada.

09.00 – 09.30  Silvia Noemí López - Development of the sterile insect technique to control the tomato moth Tuta absoluta (Lepidoptera: Gelechiidae)

09.30 – 10.00  Cherif A. Harbaoui Kh. Grissa Lebdi K – SIT against Tuta absoluta in Tunisia

10.00 – 10.30  COFFEE BREAK

10.30 – 11.00  Des Conlong - The development of an SIT programme for the sugarcane stalk borers Eldana saccharina Walker (Lepidoptera: Pyralidae) and Chilo sacchariphagus Swinhoe (Lepidoptera: Crambidae) in South Africa

11.00 – 11.30  Nevill Boersma - SIT for FCM: A success story

11.30 – 12.00  Invited speaker 1: Bernard Blum (Académie d’Agriculture de France) - Regio Biocontrol Project: a new European consortium of industry and regional governments developing area wide integrated pest management approaches including the use of sterile insect technique to control codling moth

12.00 – 12.30  Discussion

12.30 – 14.00  LUNCH
SESSION 2: Chairperson: M. Chen

14.00 – 14.30  Gregory S. Simmons, Michelle Walters and Hannah Nadel - Update on the status of Area-Wide Moth SIT programs in the United States. Prospects for new targets and strategies to ensure continued success

14.30 – 15.00  Invited speaker 2: Luc Brodeur - Sterile onion maggot project in Quebec, Canada (via Skype)

15.00 – 15.10  Invited speaker 3: Cary Gates (Flowers Canada) - Update on sterile Leafminer project.

15.10 – 15.30  COFFEE BREAK

15.30 – 16.00  Carpenter, J.E., Blomefield, T., Hight, S.D., Hood-Nowotny, R., Wohlfarter, M., and Stenekamp, D. - Laboratory and field bioassays to monitor quality and performance of moths in a sterile insect release program.

16.00 – 16.30  Nancy Barradas-Juanz, Francisco Díaz-Fleischer and Diana Pérez-Staples - Sex ratio effects on mating behaviour and improvements on the larval diet and rearing method of the mahogany shoot borer (Hypsipyla grandella).

16.30 – 17.00  Discussion

17.45  Social Event

Tuesday 3 June 2014

SESSION 3: Chairperson: Diana Staples

08.30 – 09.00  Invited speaker 4: Hugh Philip – Area wide IPM to control codling moth and the reduction in pesticide sales to control codling moth.

09.00 – 09.30  Bill Woods - Barriers to the use of SIT for eradication of exotic moth incursions in Western Australia.

09.30 – 10.00  Matthew Addisson - Codling moth quality management in South Africa.

10.00 – 10.30  Discussion

10.30 – 11.00  COFFEE BREAK

11.00 – 11.30  Suk Ling Wee and Kye Chung Park - Electrophysiological response of diamondback moth to Brassica-derived volatiles

11.30 – 12.00  Maohua Chen - Population genetics of a newly invasive species, Cydia pomonella in China: basic information for SIT.

12.00 – 12.30  Discussion

12.30 – 14.00  LUNCH
SESSION 4: Chairperson: Des Conlong

14.00 – 14.30 Invited speaker 5: Haley Catton – Biological control of invasive weeds using weevils


15.00 – 15.30 Meiying Hu, Qunfang Weng, Haohao Fu, Ke Zhang - Influence of irradiation on flight ability and dispersal ability of Litchi stem-end borer Conopomorpha sinensis Bradley.

15.30 – 16.00 Discussion

16.00 – 16.30 COFFEE BREAK

16.30 – 17.00 Ally Harari, Adi Sadeh and Hadas Steinitz - Separating male out of female pupae for SIT purposes. The case of Lobesia botrana

17.00 – 17.30 Rebecca Hood-Nowotny - Review of the isotope techniques and future ventures in moth quality control

17.30 – 18.00 R. K. Seth, Zubeda, Mahtab Zarai, Bindyia Sachdev, D. K. Rao and R. K. Bhatnagar –. Appraisal of crucial behavioural, physiological and biochemical characteristics of radio-sterilized lepidopteran pest, Spodoptera litura (Fabr.) and its progeny to establish the quality traits for a critical assessment of F1 sterility technique

18.00 – 18.30 Discussion

Wednesday 4 June 2014

08.30 – 10.00 Bus from Kelowna to Oliver

10.00 – 11.45 Visit Fairview Orchards with Jack Machial and Fairview Packers with Rick Machial.

12.00 – 13.25 Lunch at Hester Creek Winery

13.25 – 14.00 Bus to Osoyoos

14.00 – 16.00 Visit Codling Moth Rearing Facility

16.00 – 18.00 Bus from Osoyoos to Kelowna

Thursday 5 June 2014

08.30 – 10.00 Preparation of the final report of the CRP by the participants in different working groups.

10.00 – 10.30 COFFEE BREAK

10.30 – 12.30 Preparation of the final report of the CRP by the participants in different working groups.
12.30 – 14.00 LUNCH
14.00 – 18.00 Preparation of the final report of the CRP by the participants in different working groups.

**Friday 6 June 2014**

08.30 – 10.00 Discussions on final papers for the special issue in the Florida Entomologist
10.00 – 10.30 COFFEE BREAK
10.30 – 12.30 Finalisation of report
12.00 – 14.00 LUNCH
14.00 – 16.00 Presentation of the final CRP report, closure of the RCM