Sterile Insect Technology - Research and Development

A. Introduction

1. The use of sterile insects in area-wide integrated pest management (AW-IPM) programmes will continue to expand as increasing constraints are placed on the use of chemical pesticides in relation to the public's growing demand for a cleaner environment and residue-free food. In addition, the international standards that regulate global agricultural trade, e.g. the World Trade Organization's (WTO) Agreement on Sanitary and Phytosanitary Standards (SPS), are creating an environment in which area-wide approaches to the management of insect pests have a comparative advantage. Expanding globalization will also lead to the introduction of alien insect pests into new areas and the Sterile Insect Technique (SIT) can be an effective way to deal with these introductions. To counter the impact of increasing pest movement will require increased Research and Development (R&D) to develop the SIT for more species and to integrate the SIT into systems approaches that combine various risk mitigation measures to allow the export of pest free agricultural products and thus overcoming non-tariff trade barriers.

2. The use of the SIT continues to rely on the application of ionizing radiation as a means to effectively sterilize insects without affecting the ability of the males to function in the field and successfully mate with wild female insects. Ionizing radiation is the sole technology that can be used to achieve these twin goals of sterility induction coupled with effective field performance. This method is integrated with a whole suite of complementary measures that ensure success in reducing or eliminating insect pest populations. This complementarity is achieved through collaboration with other specialized organisations and, where necessary, using in-house R&D.

3. The SIT technology has been traditionally associated with programmes aimed at the eradication, i. e. the complete elimination of all individuals of pest populations; indeed it is one of the strengths of the technology that it is able to do this. However, sterile insects can also be deployed in AW-IPM programmes in other control strategies. Sterile insects can be released in a containment strategy to prevent an established pest invading a nearby area still free of the pest. An example of this is the release of sterile screwworms as a barrier in Panama to avoid re-invasion of Central America from where this pest has been eradicated. Sterile insects can also be released in a preventative strategy in pest-free high-risk areas, where there is a permanent threat of the introduction of an alien species; an example of this is the Preventative Release Programme for Mediterranean fruit fly (medfly) in California. The majority of sterile flies, which are continuously being released as part of this programme, come from the El Pino facility in Guatemala (see Figure 1), where the genetic sexing strain (allowing male-only production) developed by the Agency is being mass-reared. A fourth deployment strategy, which is considerably gaining in importance and encouraging the involvement of the private sector, is to use sterile insects as a "biological insecticide" to continuously suppress a pest

Figure 1. El Pino Guatemala, the largest mass-rearing facility in the world.

population below a level which causes economic damage without any intention of eradication of the pest population.

4. The continued expansion of the technique will also be facilitated if commercial companies can become involved and governments may decide to subcontract private companies to operate a whole programme or just individual components of a programme. The diversification described above in the use of sterile insects will help to develop commercialization. However, commercialization of the SIT has been a difficult concept to promote and establish, despite the fact that there is currently no shortage of customers who would purchase sterile insects if they were available. The use of sterile insects only for eradication of pest populations was not an attractive proposition for commercialization, but the widespread use of sterile insects for suppression, containment, and prevention programmes provides some continuity in the need for sterile insects. Commercialization will require a regulatory framework to facilitate the production, trade, shipment, and release of sterile insects.

5. AW-IPM programmes are logistically complex and management intensive; their implementation requires flexible procedures and non-bureaucratic management structures. In general, the scientists who have been closely involved with developing the technology should not be responsible for programme implementation, as other skills are needed. Also research activities should not be part of an operational programme; instead, a separate unit (but associated with the programme) is needed for problem-solving and continuous improvement of procedures; technology can always be improved. The basic components in the use of the SIT — mass-rearing and release of sterile insects — are hardly likely to change. However, as new technologies come on line and new scientific discoveries are made, R&D will continue to play a major role in improving the overall effectiveness and efficiency of the technique. The topics highlighted below could be some exciting new R&D components of future SIT programmes.

B. Improving Measurement of Insect Competitiveness

6. Success or failure of a programme that includes an SIT component is directly related to the ability of the released sterile males to effectively inseminate wild females with sperm that is competitive with normal sperm. This is generally termed "competitiveness" (see Figure 2), and it is determined by many factors related both to the treatment of a particular cohort of released insects and the developmental history of the mass-reared strain used to produce the cohort for release. These factors have negative impacts on the competitiveness ability of the released males, however, new protocols and procedures are continually being designed to minimize these negative effects. One area of active research is focused on increasing the survival of the released sterile males by providing an improved adult diet before the flies are released. An extension of the effective lifespan of the sterile insect in the field would have a significant impact on programme efficiency. However, in order to assess the effect of these changes, new protocols are being developed through R&D, which can assess competitiveness in a realistic way. These new protocols have now

Figure 2. Field cage to evaluate medfly competitiveness.

been incorporated into an internationally agreed quality control manual "Product quality control and shipping procedures for sterile mass-reared tephritid fruit flies" (FAO/IAEA/USDA 2003). The protocols involve field cage evaluations of competitiveness and in the future may include very large caged field arenas where many components of insect behaviour can be researched.

C. Colony Replacement Procedures and Filter Rearing System (FRS)

7. The artificial environment of a mass-rearing facility presents the insect with a tremendous colonization challenge, i.e. the need to adapt to the artificial biotic and abiotic conditions. Successful colonization inevitably results in the selection of adapted genotypes, which are very successful within the facility but which can impact negatively on the competitiveness of a sterile insect once it is released in the field. Most mass-rearing protocols follow the principle of large cycling colonies, whereby a proportion of the production is used for sterilization and release while the remainder is returned to maintain the production colony. In this system, over time there is an inevitable accumulation of highly selected genotypes necessitating the regular replacement of strains. Strain replacement is a major logistical exercise, and research is ongoing to improve colony-holding conditions to increase the viable life of strains under colonization. This involves assessing the effect of different levels of colonization stress on field competitiveness.

8. The Agency's success in transferring medfly genetic sexing technology to operational SIT programmes required an R&D effort to develop a protocol to maintain the integrity of the genetic sexing strain in operational programmes (Caceres et al. 2004). The filter rearing system (FRS) relies on the careful maintenance of a pure-breeding mother colony from which eggs are harvested, and following 3 to 4 generations of mass-rearing the resulting males are sterilized and released. In the FRS, no insects that have been through mass-rearing are returned to the mother colony, and therefore there is no accumulation of highly selected genotypes in the colony.

9. The FRS needs to be further developed in view that it can also make a major contribution to improving the overall competitiveness of mass-reared strains as the mother colony can be kept under more natural environmental conditions, at reduced adult and larval densities, and with reduced selection pressure for adapted genotypes. In addition, a more natural environment, preferably under greenhouse conditions with hosts and natural light, could help address the major problem of loss of irritability and predator-evasion behaviour in mass-reared insects (Hendrichs et al. 2006). Significantly more research on the FRS is required to achieve adoption in mass-rearing facilities so that the viable life of a mother colony is extended and strain replacement becomes a much simpler procedure and can be done without major disruption of production. It is anticipated that the size of a mother colony for a large mass-rearing facility will be only about several thousand individuals and this colony can be easily replaced during one generation.

D. Sterile Male Performance

Figure 3. Applying juvenile hormone to a fruit fly.

10. The application of hormonal, nutritional, semiochemical supplements or other bioactive materials to sterile males before their release can improve their competitiveness in the field (see Figure 3). The application of juvenile hormone mimics to emerging sterile male Anastrepha fruit fly species have been shown to significantly advance sexual maturation by 5 to 7 days. This acceleration of reproductive development is crucial for improved SIT application as normally a majority of sterile males are lost to predation and other causes before they reach sexual maturity (Teal et al. 2000). Research is ongoing to test these hormones in other pest species and to develop practical applications under operational conditions.

11. Nutritional supplements are critical for sexual development and signalling in some species, and adding protein to the diet fed to sterile males prior to release often significantly increases competitiveness (Shelly et al. 2002a, Yuval et al. 2002). Gut bacteria are also important in fly nutrition and mass-rearing may even promote non-beneficial or harmful bacteria. Thus the provision of a diet that contains beneficial gut micro-organisms, is an area with much potential for further R&D to improve sterile male competitiveness (Niyazi et al. 2004).

12. Some species that are attracted to natural attractants sequester these as precursors for pheromone (sexual attractants) production into their systems, and subsequently release them during courtship and mating. Such components fed to sterile males of Bactrocera spp. before release can significantly improve competitiveness (Tan 2000). The competitiveness of Mediterranean fruit fly males is similarly considerably enhanced by exposure of pre-release sterile males to ginger root oil and citrus peel oils (Papadopoulos et al. 2001, Shelly et al. 2002b). Even exposure to vapour has this effect enabling the development of an “aromatherapy” protocol that will facilitate application in fly emergence facilities (Shelly et al. 2004a). Researchers in a number of countries are involved under an Agency Coordinated Research Project in expanding the understanding of these phenomena in order to develop direct application in SIT programmes.

13. There is also the potential to use sterile males as carriers of various bioactive materials (Knipling 1979). In auto-dissemination, sterile males would be inoculated with electrostatically charged powder formulated with entomopathogens or slow-acting insecticides, which would be spread throughout the pest population through intraspecific interactions, primarily during mating (Howse 2005). In auto-confusion, sterile male Lepidoptera (moths) would carry on their bodies pheromone particles that attract wild males. These particles would then be transferred among the wild males, resulting in increased mating disruption, i.e. interfering with wild males finding wild males for mating (Knipling 1979, Howse 2005). A further possibility is simultaneous mating disruption of a moth pest and SIT application against a second pest in the same ecosystem. Sterile male medflies, inoculated with the moth pheromone would cause mating disruption in the moth population while at the same time suppressing the medfly population. There are probably many other possibilities of applying bioactive materials to emerging sterile males, but so far these are only theoretical, and much research is required to explore the various possibilities.

E. Reducing the Radiation Dose

14. Radiation is one of the contributing factors to reduced competitiveness of sterile insects; the higher the radiation dose, the more the competitiveness of the insect can be compromised. In most programmes, the radiation dose used produces almost full sterility when measured in a laboratory. However, measuring sterility in a laboratory does not take into account factors such as field competitiveness that, in the end, will determine sterility in the wild females. If a lower dose is used, a more competitive insect is released, which at the end induces more sterility in the wild population (Toledo et al. 2004). Therefore, an optimum radiation dose for each particular pest species and strain should be identified (Mehta and Parker 2006). This will involve further R&D to address the different parameters and possibilities, including the use of different gaseous environments for radiation and the use of new sources of X-rays, e.g. electron beam.

F. Shelf-Life and Shipment

15. In many areas of the world, pest problems are seasonal, and sterile insects need only be released during specific times of the year. In insect species that have a diapause (a quiescent state to survive adverse winter or other conditions), physiological research is needed to allow manipulation of the diapause to allow continuous mass-rearing. On the other hand, this behavioural trait can also be manipulated to store insects during the time when they are not required for release and this trait can be exploited when shipping sterile insects (Bloem et al. 1997). However, many tropical and subtropical pest species have no diapause, and these insects cannot be stored. Recent developments in the cryopreservation of insect embryos (Leopold 2005) may provide a procedure for stockpiling eggs.

16. Sterile insects, usually in the late pupal stage, are often transported over long distances and some form of cooling and/or anoxia is used to prevent adult emergence during transit. This procedure can reduce insect quality as the pupae are shipped in cardboard containers containing cool packs to maintain a low temperature. Durable equipment needs to be designed that would maintain the correct temperature and atmospheric conditions, and increase the safety of insect shipments. Research has also resulted in procedures to ship fertile eggs from egg production facilities to satellite rearing facilities that rear, sterilize, and release sterile adults. The egg reception facilities do not maintain large adult colonies for egg production, and this greatly simplifies their operational protocols. Research has demonstrated that Mediterranean fruit fly eggs can be shipped over long distances for extended periods of time without losing their viability and affecting the quality of the insects that are subsequently mass-reared. This concept is now in operation in the Moscamed Programme in Guatemala and Mexico, where heat-treated eggs from a genetic sexing strain maintained at the mass-rearing facility in Guatemala are shipped daily to the male-rearing facility in Tapachula, Mexico (Tween, 2004).

G. Field Monitoring and Release

17. The area-wide approach to pest management requires that sterile insects be distributed as adults over large areas, which is usually done by aircraft. The current practice is to release sterile insects via an auger from a chilled container in the aircraft. The logistics of this procedure for very large numbers of insects are daunting, given that insect quality must be maintained at the highest possible level. The technology to hold insects at a low temperature for several hours in an aircraft, and to achieve the required distribution over a release area, has greatly improved. Nevertheless, there is still a major concern about the negative impact on insect quality from the stress of low temperature and high insect density in the release machine. Research is ongoing to develop new systems that are based on cryogenics (liquid CO2 to make dry ice pellets) as the cooling component. The objective is to simplify maintenance, eliminate the high electric load on the aircraft, and allow greater control over temperature and humidity, thus minimizing damage to the sterile insects (Tween 2005). Computer software linked to a satellite-guided aerial navigation system is programmed to deliver to the field an adjustable number of sterile insects (as needed in each release block) and to turn off the release machine when the airplane is outside the target blocks. The performances of the pilot, aircraft, and machine are recorded, and can be analysed after each flight.

18. Other applications of Global Positioning System (GPS) and geographic information systems (GIS) are being developed to dramatically increase the accuracy and ease with which insect populations can be monitored before, during, and after the implementation of a programme (see Figure 4). This increase in precision will enable much better use of programme resources, and rapid decisions to modify programme activities. The use of bar-coded traps, and the ability to enter field data directly into hand-held computers for rapid downloading at the field centre, will increasingly make a major contribution to the accuracy and accessibility of data, and enable managers to monitor the efficiency of trapping personnel. Improved aircraft navigation systems also facilitate the accuracy of the sterile fly releases.

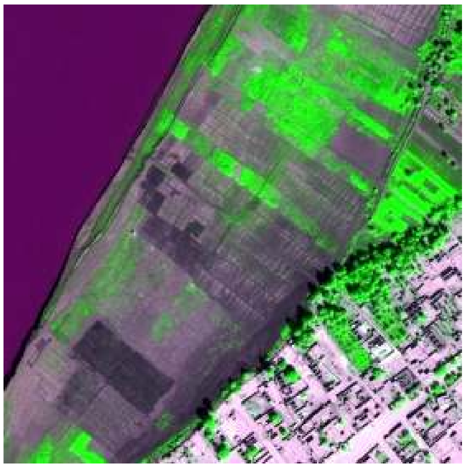

Figure 4. Quick Bird images from Northern Sudan.

H. Population Genetics

19. Population genetics using DNA analysis is now a very powerful tool to study the structure of field populations of insects. Information on this can greatly help in determining the origin of pest introductions and in identifying potential target populations by assessing gene flow between populations and in so doing infer the degree of migration between adjacent populations. In tsetse, where some species have very wide distribution, this technology will be very important to identify isolated populations and a Coordinated Research Project (CRP) on this topic has now been completed. The results have shown that there is much sub-structuring within populations of certain species thus suggesting that relatively well isolated populations can be identified for SIT intervention. The New World screwworm, Cochliomyia hominivorax, has been eradicated from all of Central America through the use of SIT in an integrated approach and a sterile fly barrier system is now in place in

Panama to prevent re-invasion of this pest from South America. The pest is still present in several islands in the Caribbean and in South America, where it is endemic, and several countries are interested to assess the feasibility of using SIT to eradicate the pest. A current CRP is addressing this possibility by assessing the genetic relationships of screwworm populations in South America and in the Caribbean.

20. Although modern DNA analytical tools provide very powerful ways to assess gene flow between pest populations, they need to be accompanied by mating compatibility evaluations. The lack of gene flow between two populations clearly indicates that they are isolated. However, this does not mean that mating could not take place between them if the populations were brought together or that a particular colony producing sterile males would not be effective against them both. That is why mating compatibility protocols are needed for the different species that are amenable to SIT application.

I. Modern Biotechnology

21. Techniques used in modern biotechnology now enable genes to be routinely introduced into the germ-line of many pest species (Robinson et al. 2004). This type of transgenic technology may eventually benefit operational SIT programmes in three areas: genetic sexing, marking and sterilization.

22. Genetic Sexing: The medfly genetic sexing technology successfully transferred to operational SIT programmes is based on classical Mendelian genetics. These strains are only 50% fertile, females show reduced viability and they are not transferable to other species. These factors have encouraged the search for molecular approaches to genetic sexing. Molecular approaches could either lead to female death or the transformation of females into males. In Drosophila, a tetracycline repression system has been used to construct female killing systems (Heinrich and Scott 2000, Thomas et al. 2000), but as yet such a system has not been used in a pest insect. Transforming females into males requires a detailed knowledge of sex determination, but fortunately in Diptera, there is quite a lot of conservation at the molecular level of many of the genes involved in this process. Using Drosophila DNA sequences as probes, various sex-determining genes have been cloned from the medfly, and one of these genes transformer (tra) has been the target for transforming females into males by injecting double-stranded RNA (dsRNA) for part of the tra gene into embryos (Pane et al. 2002). The dsRNA prevents expression of the tra gene. Following the injection of dsRNA into medfly embryos it was possible to demonstrate the transformation of female embryos into functional fertile males (Pane et al. 2002).

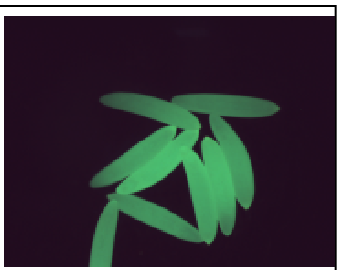
Figure 5. Medfly eggs expressing GFP

23. Marking: Sterile insects for release are usually marked with a fluorescent powder to distinguish them from wild insects. Genes encoding fluorescent protein markers, i.e. green fluorescent protein (GFP) (see Figure 5) and red fluorescent protein (DsRed) can now be introduced into pest insects to provide a more secure and easier system for identifying sterile insects. Generalized expression of the proteins can be obtained and the protein can be observed in dead insects. Particular strains can show very strong levels of expression, but the effect of this on the competitiveness of the insect is unknown.

24. Sterilization: Transgenic strains have been produced in Drosophila that induce embryonic lethality in eggs fertilized by released fertile transgenic males carrying a dominant lethal gene. The system must be conditional in some way so that efficient mass-rearing can be carried out. This is done by the addition of antibiotics to the larval diet that shuts down the lethal gene (Heinrich and Scott 2000, Thomas et al. 2000). Recently, in the medfly, similar strains have been produced (Gong et al. 2005). However, these strains will require further refinement because they are not completely sterile in matings with wild females, and the majority of the lethality occurs in the late larval stage. These types of strain are at a very early stage of laboratory development and much R&D is needed before they can be evaluated in any sort of operational programme.

J. SIT Research for Malaria Mosquito Control

25. A new project was initiated in 2004 at the Agency to assess the feasibility of using the sterile insect techniques (SIT) for mosquito control. Currently, research is focused on Anopheles arabiensis which is the second most important vector of malaria in Africa. The project will seek to develop innovative ways of mass-rearing large numbers of mosquitoes, methods to eliminate females so that only sterile males will be released, and appropriate ways to sterilize male mosquitoes. The project also has a field component to collect and evaluate base-line data in potential field sites where feasibility studies can be carried out in the future. Initial field work has been carried out in Northern Sudan and on the island of Réunion. A coordinated research project on development of standardized mass-rearing systems for male Anopheles and Aedes mosquitoes has been launched, and is anticipated to last until 2010. It is intended to improve mosquito mass-rearing efficiency to a level where it can be applied in field pilot projects.

K. Conclusions

26. Global trends towards a cleaner agriculture, less aggressive pest control and increasing international trade will encourage the development of area-wide approaches integrating the release of sterile insects. Continuing R&D will permit the removal of some key technical and scientific constraints and the introduction of new technological innovations will identify new target pests and improve efficiency of current programmes. However, these conditions will not guarantee successful implementation of the SIT as part of AW-IPM programmes. Effective management and organization are the key factors. Since the area-wide approach also depends on the cooperation and participation of all stakeholders in the target area, good communication is essential with attention being paid to political, socio-economic, and environmental sensitivities.

27. A major unexploited opportunity is the integration of sterile insect release with the release of biological control agents. When key pests are treated with insecticides, biological control becomes difficult. However, when a key pest can be managed using sterile insect release then mass-reared biological control agents can also be used. This would indicate that the current biocontrol industry commercially selling natural enemies (ANBP 2005, IBMA 2005) and the SIT appear to be natural allies. The biocontrol industry already has the technical “know-how” to manage the mass-rearing, quality control, handling and shipping of insects; and adding sterile insects to their products could

provide a complete "biological package". A pioneer in this approach is Bio-Bee in Israel (Bassi 2005). Radiation induced sterility is also being researched as part of an Agency CRP in the production and use of biological control agents (Greany and Carpenter 2000) involving the use of sterile insects as hosts/prey/vectors for parasitoids/predators/pathogens.

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