

# THE STERILE INSECT TECHNIQUE AS A COMPONENT OF SUSTAINABLE AREA-WIDE INTEGRATED PEST MANAGEMENT OF SELECTED HORTICULTURAL INSECT PESTS

Marc J.B. Vreysen\*, Jorge Hendrichs  
and Walther R. Enkerlin

Insect Pest Control Section, Joint FAO/IAEA Division of Nuclear Techniques in  
Food and Agriculture, Department of Nuclear Applications, International Atomic  
Energy Agency, Wagramerstrasse 5, A-1400 Vienna, AUSTRIA

\*Corresponding author: e-mail: M.vreysen@iaea.org

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## A B S T R A C T

The indiscriminate use of broad-spectrum insecticides has caused major problems with pest resistance, residues in food, environmental contamination, outbreaks of secondary pests, and reductions in populations of beneficial insects. This results in increased demands for pest control methods that are both efficient and friendly to the environment. The sterile insect technique (SIT), applied as part of an area-wide integrated pest management approach (AW-IPM), offers considerable potential and has been used with great success against major pests of agricultural importance to establish pest-free areas (eradication), areas of low pest prevalence (suppression) or to maintain areas free of the pest through containment or prevention.

This review describes recent advances in the SIT, with practical examples of how SIT programs have been designed and carried out to control different insect pests of agricultural importance, especially the Mediterranean fruit fly, the codling moth, the date moth, and the cactus moth. Topics discussed include:

- technological breakthroughs in production and delivery systems;
- recent advances in insect genetics;
- the development of genetic sexing strains;
- cost-effectiveness;
- the role private companies can play; and
- the potential long-term direct and indirect economic benefits associated with the successful implementation of AW-IPM programs with an SIT component.

In view of the increasing demand of environmental-friendly control tactics, it is anticipated that the SIT, as part of area-wide pest management approaches, will increasingly gain in importance in the years/decades to come.

**Key words:** sterile insect technique, area-wide integrated pest management, Lepidoptera, fruit flies, eradication, suppression, prevention, containment

## **INTRODUCTION: THE PROBLEM**

The population of the world is increasing by 80 million people per year. This means that agricultural output has to almost double within the next thirty years to meet the growing demand for food (Hendrichs, 2000). Despite the annual use of three million tons of pesticides worldwide and a 5% increase annually, 3.7 billion humans still suffer from malnourishment (Pimentel, in press).

The indiscriminate use of broad-spectrum insecticides has caused major problems with pest resistance, residues in food, environmental contamination, outbreaks of secondary pests, and reductions in populations of beneficial insects. These developments have, combined with the mounting awareness of the general public increased demands for pest control methods that are both efficient and friendly to the environment.

### **1. AREA-WIDE INTEGRATED PEST MANAGEMENT (AW-IPM)**

The area-wide approach to integrated pest management (AW-IPM) targets *entire* insect populations within a delimited geographical area (Klassen, 2005). It remains undoubtedly the most rational way to control major agricultural insect pests. Although the approach is area-bound, management intensive and requires the coordinated involvement of farmers' associations, local governments, and regional authorities, AW-IPM results in more sustainable, long-term insect pest control (Lindquist, 2000). The emphasis of the approach is on preventing the existence of infestation from which recruits can re-establish damaging densities of the pest population (Klassen, 2005). Knipling (1972) pointed out the implications of leaving small relic pockets where the pest continues to reproduce: More pest individuals are produced if 1% of the total population were allowed to reproduce without control, while 100% control were applied to the other 99% of the population, than if only 90% control were imposed uniformly on the total population.

Traditional approaches to pest management are applied on a field-by-field basis. They focus on protecting a particular field or orchard, and do not deal with the pest insects in abandoned orchards, wild hosts and backyards. Traditional pest management, particularly since the advent of modern insecticides since the mid-20th century, requires minimal planning, and is applied reactively by individual farmers. The techniques used in traditional pest management are simple and can be carried out by the farmers themselves.

AW-IPM, on the other hand, is based on preventive management of entire pest populations. It requires careful long-term planning and coordinated organization to successfully implement the strategy. AW-IPM is usually

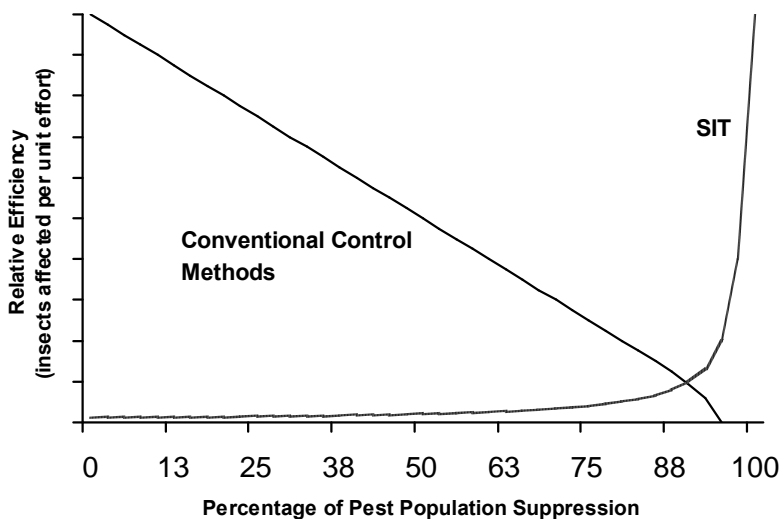
carried out using advanced techniques such as geographic information systems, remote sensing, mathematical models and population genetics requiring a high degree of specialized expertise (Klassen, 2005).

One very effective control strategy that can be integrated in AW-IPM programs is the Sterile Insect Technique (SIT).

## 2. SIT AS A COMPONENT OF AW-IPM

The concept of controlling, managing and eliminating insect pests by manipulating reproduction was conceived as early as the 1930s (Knipling, 1955). There was a major breakthrough in the 1950s, when laboratory experiments showed that both female and male New World screwworm flies (*Cochliomya hominivorax* (Coquerel)) could be rendered sterile by exposure to X-rays without excessive loss in lifespan and competitiveness (Bushland and Hopkins, 1953).

Simple theoretical models have shown that the sustained, sequential and systematic release of sterile male insects could completely eliminate a target insect population within a few generations (Knipling, 1955). SIT involves the production of large numbers of the target insect in specialized breeding facilities, sterilization of one or both sexes, and the sustained and systematic release of the sterile individuals in numbers large enough to effectively compete with the wild pest population.



**Figure 1.** Efficiency of conventional control methods and the Sterile Insect Technique (SIT) in area-wide integrated pest management

The SIT, as a biologically-based genetic control method has many advantages i.e. it is non-intrusive to the environment, does not adversely affect non-target organisms, acts inversely density dependent (Fig. 1) and can easily be integrated with other biological control methods such as parasitoids, predators and pathogens.

However, the SIT can be effectively used only if:

- the target pest is present in low numbers;
- the biology and ecology of the target pest is known in great detail;
- the target pest can be reared in large numbers;
- the sterile individuals can be efficiently released and monitored; and
- the releases have to be applied on an area-wide basis and cannot be used by an individual farmer.

Furthermore, SIT requires intensive management and long-term commitment.

The SIT was first used in 1954 on the island of Curaçao in the Netherlands Antilles to control the New World screwworm fly. After sterile males had been released for six months, the pest was completely eliminated (Baumhover et al., 1955). This programme ultimately culminated in the eradication of 1 – plum nursery; 2 – nursery of *Prunus divaricata* seedlings; 3 – nursery of *Prunus mahaleb* seedlings; 4 – apple orchard; 5 – apple scion plantation; 6 – plum orchard

the New World screwworm fly from Mexico, Central America and the southern United States (Wyss, 2000). This was the largest and most successful SIT-based eradication campaign ever undertaken against a major insect pest.

The SIT has also been used to successfully control other major horticultural pests, including:

- the Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) in Mexico, Chile, Western Australia, Argentina, Chile, Israel, Jordan, the USA and South Africa;
- the melon fly (*Bactrocera cucurbitae* (Coquillett)) on the Okinawa Islands of Japan;
- the Queensland fruit fly (*Bactrocera tryony* (Froggatt)) in Australia;
- the Mexican fruit fly (*Anastrepha ludens* (Loew)) in northern Mexico; and
- the West Indian fruit fly (*Anastrepha obliqua* (Macquart)) in northern Mexico (Cayol et al., 2002).

### 3. STRATEGIC APPROACHES

AW-IPM with an SIT component has been used with great success to control several major horticultural pests following four major strategies: eradication, suppression, containment and prevention (Hendrichs et al., 2005).

### 3.1. Eradication

One example of an AW-IPM programme achieving eradication is the campaign against the melon fly on the Okinawa Islands. The melon fly was first discovered in 1919 in the Yaeyama Islands. By 1980, it had spread to most of the southern islands of Japan. On the Okinawa Islands, the fly not only caused significant damage to a wide variety of hosts, it also prevented the export of crops to areas free of infestation. This incited the Japanese government together with local prefecture governments to launch a program to eradicate the melon fly from the southwestern islands of Japan. Following a pilot program on Kume Island, which confirmed that the proposed strategy could be successfully applied a full-scale AW-IPM campaign with an SIT component was launched in 1984. A facility for producing sterile melon flies was established with an initial production capacity of 30 million sterile male flies per week. By 1986, production had increased to 200 million sterile males per week. The fly population was reduced to 5% of its original level using the male annihilation technique. This was followed by the dispersal of sterile males by helicopter. In 1993, the melon fly had been completely eradicated from the Okinawa Islands (Koyoma et al., 2004).

### 3.2. Suppression

An example of the successful suppression of a major lepidopteran pest using an AW-IPM approach is the campaign against the codling moth (*Cydia pomonella* (L.)) in British Columbia, Canada. The codling moth is the most important pest of pears and apples in British Columbia (Bloem and Bloem, 2000). From 1976 to 1978, a pilot program was carried out in the Similkameen Valley. This pilot program demonstrated that the codling moth could be effectively controlled by using a combination of chemical agents and releases of sterile moths (Poverbs et al., 1982). In 1995, a full-scale program was initiated in the Okanagan Valley. Since then, the apparent density of the wild codling moth population has dramatically decreased in the first intervention zone. The number of adult moths caught in pheromone traps dropped from an average of 2.5 - 13.0 moths per trap per week in 1995 to an average of 0.08 moths per trap per week in 2000. The proportion of orchards with no detectable codling moth damage increased from 42% in 1995 to 91% in 2000. The amount of organophosphate insecticides purchased decreased from 18,903 kg in 1991 to 3,403 kg in 2001 (Bloem et al., 2005).

A series of technological improvements have optimized the rearing procedures for the Mediterranean fruit fly and have reduced the overall cost of the SIT component making the SIT competitive with other more traditional control tactics. As a consequence, most AW-IPM programs with an SIT component against the Mediterranean fruit fly currently focus on suppression, rather than eradication. Examples include the suppression programs:

- in the Hex valley in South Africa (Barnes et al., 2004);
- in the Arava/Araba valley in Israel and Jordan (Cayol et al. 2004);
- on the Cap Bon in Tunisia;
- on the island of Madeira, Portugal (Dantas et al., 2004); and
- in the province of Valencia in Spain.

### **3.3. Containment**

In 1976 and 1977, Guatemala, Mexico and the United States signed agreements that culminated in the establishment of the “Programa Moscamed”. The aim of this project was to contain the Mediterranean fruit fly in Guatemala and to prevent it from spreading into Mexico and the United States using an AW-IPM approach with an SIT component, thereby protecting the horticulture industry in all three countries.

In the initial years (1977-1982), the program eradicated the Mediterranean fruit fly from 6,400 km<sup>2</sup> it had already invaded in Chiapas, Mexico integrating legal measures (quarantine), chemical, mechanical, cultural and genetic (SIT) methods (Villaseñor et al., 2000). Since then, a barrier of sterile flies has been successfully established, which has prevented the pest from spreading northward (Enkerlin, 2005). As a result of the medfly-free status,, exports of horticultural products from Mexico have increased to over 3.5 billion US dollars a year and exports of fresh vegetables and fruits have risen by 80 and 90%, respectively. The program can rely on the availability of over 2.5 billion sterile male flies produced each week at the El Pino mass-production facility in Guatemala (Tween, 2004)

### **3.4. Prevention**

Until 1980, periodic outbreaks of the Mediterranean fruit fly in California and Florida were eliminated mainly with unpopular aerial malathion-bait sprays, often over urban areas. This method was costly as exemplified by the outbreaks in 1980 and 1982, which cost more than 100 million US dollars to bring the situation under control. Subsequent outbreaks were addressed only on a local basis, with limited success, prompting the initiation of an eradication programme in 1994, using an area-wide SIT approach.

Because this program was both successful and cost-effective, a permanent preventive release program covering the entire Los Angeles Basin was initiated in 1996 (Dowell et al., 2000). Since then, more than 400 million sterile males have been released each week over an area of 2,489 square miles. This program costs about 15 million US dollars a year, which compares very favorably with the average annual cost of reactively controlling outbreaks before the program was implemented (30 million US dollars).

## 4. NEW DEVELOPMENTS IN AW-IPM WITH AN SIT COMPONENT

Science, technology and methods development are instrumental to improve techniques for insect detection, monitoring and suppression, to increase the quality and quantity of the insects reared and to advance techniques to deliver the sterile insects as close as possible to the target virgin females. In the past decade, numerous new developments have significantly improved various aspects of AW-IPM with an SIT component for selected species of fruit flies and lepidopteran pests. A detailed review of all these developments is beyond the scope of this paper, but the following selection highlights some of the progress made.

### 4.1. The Mediterranean fruit fly

#### 4.1.1. Genetic sexing strains

The introduction of sterility in a wild pest population is the underlying principle of the sterile insect technique. To induce sterility in the wild population of Mediterranean fruit flies, the release of both males and females is less effective than the release of males only (McInnis et al., 1986; Rendon et al., 2004). Sterile females are not required for the SIT component, as they do not contribute to the transfer of sterility into the native population.

In 1981, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated activities to develop genetic sexing strains of the Mediterranean fruit fly that would allow the release of exclusively males.

The first generation of genetic sexing strains used a sex-linked color mutation that was expressed during the pupal phase. The males were brown, whereas the females were white. However, all the female larvae still had to be reared up to the pupal phase, which was very costly. Furthermore, pupae had to be sorted using seed sorters that were not only expensive, but had complicated mechanics and gave only 95% accuracy (Cáceres et al., 2004).

By using a new temperature-sensitive lethal mutation (*tsl*), all females can be eliminated by exposing the eggs to 35°C for 24 hours (Franz, 2005). Although the production costs are about the same for the *tsl* train as for the standard bi-sexual strains (Cáceres, 2002), marking, irradiating, transporting and releasing *tsl* individuals is considerably less expensive (Franz, 2005). Moreover, these genetic sexing strains are stable under mass rearing conditions and the release of males-only avoids the damage caused by sterile female stings in the fruit (reducing its economic value), increases the safety of the operations (no accidental releases of females possible), simplifies the monitoring (using female attractants, only wild females are trapped and there is no trapping out of sterile males), increases bio-safety and the effectiveness

of the released sterile male flies resulting in a higher rate of induced sterility (Hendrichs et al., 1995; Cáceres et al., 2004; Rendon et al., 2004).

Recently, mathematical models have demonstrated that male-only releases result in relative more efficient sterile insects as compared to programmes releasing both sexes, taking into account factors such as reduced sterile sperm quantity/quality with re-mating and with ageing and incomplete re-distribution of the sterile males with the fertile insects (Vreysen et al., 2006).

#### **4.1.2. Improved production methods**

Adapting to the artificial environment of a mass-production facility presents a great challenge to the insects. They are subjected to considerable selection pressures and unwanted behavioral changes might be introduced that can impair the efficiency of the release program. This is especially true for fruit flies, which have complex courtship behaviors and a mating system based on female choice. Any minor change in their courtship behavior will put the mass-produced sterile males at an immediate disadvantage when they are released into the natural habitat.

A filter rearing system has been developed to address these problems. The system allows the mother colony to be maintained under more natural environmental conditions, with lower densities of adults and larvae and reduced selection pressures (Fisher and Cáceres, 2000). The mother colony is checked at every generation so that undesirable individuals can be eliminated. Eggs from the mother colony are used to produce a large colony following 3-4 generations of amplification. The amplified colony produces eggs, which are heat-treated to kill off the females. The males are then sterilized and released (Cáceres et al., 2004).

In this system, mass-produced insects are not returned to the mother colony, which prevents the accumulation of highly selected genotypes. Strain replacement is therefore much simpler and can be performed without disrupting the production process. This procedure is now widely used to introduce new genetic sexing strains of the Mediterranean fruit fly into production facilities (Robinson and Hendrichs, 2005).

#### **4.1.3. Quality control and management**

To ensure that an AW-IPM program with an SIT component is successful, sterile males have to effectively compete with wild males in locating and mating with wild virgin females (Vreysen, 2005). In the past, many programs relied on a strategy of “overkill” (Krafsur, 1998), but lately, more emphasis is being placed on insect quality rather than quantity (Itô and Yamamura 2005). Traditionally, the quality of the insects released was assessed under laboratory conditions rather than under more natural conditions either in large field cages or even in the natural habitat itself. It was often difficult to interpret the results



because the mass-produced insects were not given the opportunity to display their full behavioral repertoire (Cayol et al., 1999). Furthermore, when mass-produced insects were tested in small cages in the laboratory, they were often more competitive than their wild counterparts. For example, this was observed during the melon fly program on the Okinawa Islands (Iwahashi et al., 1983). As a result, quality of mass-produced fruit flies is increasingly assessed in large field cages or in the open field, where the mass-produced males are allowed to compete with wild males (FAO/IAEA/USDA, 2003; Rendon et al., 2004; Vreysen, 2005).

The expansion of existing mass-rearing facilities over the past years (Hendrichs, 2000) has triggered an increased demand for sterile Mediterranean fruit flies. This has been accelerated by the availability of genetic sexing strains and some facilities have regularly started exporting sterile fruit flies and strains to facilities in other countries. To ensure effective control, the males released have to be sexually compatible with the wild fruit flies living in the target area. Studies conducted in large field cages have shown that Mediterranean fruit flies from nine countries on five continents have not yet evolved specific sexual behaviours which indicate incipient pre-mating isolation mechanisms among local natural populations (Cayol et al., 2002). Therefore, special strains of the Mediterranean fruit fly do not have to be developed and produced to target the wild populations in specific geographical areas.

The importance of these studies prior to embarking on an operational programme is exemplified with the South American fruit fly (*Anastrepha fraterculus* (Wiedemann)). Compatibility studies indicated that there were mating barriers among different local populations, sometimes to the point that some populations could be considered to be crypto-species. This means that different strains have to be produced to target the wild populations in specific geographical regions of South America (Vera et al., 2006).

Therefore, before implementing an AW-IPM program with an SIT component, it is essential to carry out extensive compatibility studies.

#### **4.1.4. Sterile male performance**

The performance of sterile male fruit flies has been greatly enhanced by administering hormonal, nutritional and semio-chemical supplements to post-teneral sterile males before release. These chemicals may partially offset the loss in quality that often occurs during colonization, mass-production and irradiation.

After hormone treatments with juvenile hormone mimics, emerging sterile males of *Anastrepha* spp. reach sexual maturity sooner and can mate five to seven days earlier than untreated males. This improves the efficiency of SIT because a significant proportion of the sterile males is usually lost to predation

and other causes before they reach sexual maturity. Similarly, adding nutritional supplements such as proteins to the diet of sterile males before they are released often increases sexual performance. Moreover, hormone treatments and protein-enriched diets act synergistically and can increase the competitiveness of the sterile males many times more than either hormone treatments or protein-enriched diets alone (Teal et al., in press). Hormone treatments and nutritional supplements are affordable and easy to incorporate into protocols followed at fly emergence facilities. Because the sterile males can be released earlier, the costs associated with maintaining them before they are released are lower.

Semio-chemical supplements can also be used to greatly enhance mating competitiveness. For example, *Bactrocera* spp. are attracted to natural chemicals and use them as precursors in pheromone biosynthesis. These pheromones are later released during courtship. When fed to sterile males before release, semio-chemical compounds not only improve mating competitiveness, but also act as potent allomones which deter predators. Furthermore, sterile males which have been fed semio-chemicals before release are far less attracted to male-annihilation traps than their wild counterparts. Releasing sterile males and using male-annihilation traps at the same time could theoretically lead to a “male replacement” strategy (Robinson and Hendrichs, 2005)

Likewise, exposing wild or mass-produced *C. capitata* males to ginger root oil, citrus peel oils, or even the vapors of these oils can also greatly enhance their performance. This has great potential in increasing the cost-effectiveness of deploying sterile males, thus enabling the development of an “aroma therapy” that will facilitate application in fly emergence facilities in operational programmes (Robinson and Hendrichs, 2005).

#### **4.1.5. Improved sterile male release systems**

In the production facility, no effort is spared to produce high quality insects in adequate numbers for release in the target zone. Obviously, the sterile individuals should be dispersed with maximum care in order to minimize losses in quality. Fruit flies are almost always released from fixed-wing aircraft or helicopters. This is the most cost effective way of systematically releasing sterile individuals over large geographical areas. Early release methods used paper bags, which were ripped open by hand or by a hook in the release chute before being ejected from the aircraft. The main advantage was that no complex release equipment was needed. However, damage due to the collapse of the bags, or differential differences in the ambient temperature depending on the positioning in the plane were often detrimental to the flies. In addition, the empty bags littered the target site (Tween, 2004).

More sophisticated systems based on the release of chilled adults have since then been developed. In the first generation of chilled release systems, a cooling unit housed in the aircraft provided a current of cool air to immobilize the adult flies. A screw auger transported the flies to the release chute, from which they were ejected by suction through tubes in the belly of the aircraft. Once exposed to the warm air outside the aircraft, the flies revived before they reached the ground.

A new release system based on cryogenics was developed for use in the Programa Moscamed in Guatemala and Mexico. Dry ice pellets were used as the cooling agent. Temperature and humidity were carefully controlled and up to 25 million sterile males could be released in a single flight. The system is designed in such a way as to deliver the fly outside the aircraft with the minimal possible damage due to a careful balance between gravity-mechanical-suction feed to allow for a free flow of the flies. Based on the proportion of flies recaptured in follow-up studies, not only was survival higher with this new method, but the condition of the flies reaching the target area was also better.

The program currently uses the highly dependable turbine Cessna Grand Caravan, approved for single pilot operation that can release 50 million flies on each flight. The aircraft has a seven-hour range, is capable of slow flight, can land and take off on short mountain airstrips, has fixed landing gear, and requires low maintenance (Tween, in press). Furthermore, a sophisticated AgNav navigation system provides accurate guidance to the pilots and also monitor temperature, humidity, fly release rate, altitude, speed and flight time. All information is recorded and can be downloaded after the flight for detailed analysis.

#### **4.1.6. Female attractants**

AW-IPM programs, especially those that integrate the release of sterile males, require efficient monitoring methods that can detect changes in the movement, distribution, density and structure of the target wild population (Vreysen, 2005). Monitoring is costly, so when selecting monitoring methods, it is necessary to wisely use the resources available while maximizing the quality of the data collected.

Adult Mediterranean fruit flies have traditionally been monitored by sampling fruits and by deploying traps which use a combination of olfactory and visual cues to attract the target individuals. Traps baited with trimedlure, a male parapheromone, have been the most commonly used sampling tool. However, trapping large numbers of sterile males reduces the effectiveness of SIT programs and requires tedious sorting of wild and sterile males. Trimedlure traps have sometimes been used together with traps containing proteinaceous baits that attract both males and females. However, proteinaceous bait traps are not very selective. Furthermore, the attractant has

a short field life, and the traps are difficult to maintain because the attractant used is in liquid form.

The development of a highly efficient synthetic female attractant, composed of the chemicals ammonium acetate, putrescine and trimethylamine, used in male-only release programs, opened new avenues for more efficient and accurate monitoring activities (IAEA, 1999). The commercial formulation of this attractant has a field life of six to eight weeks, far longer than the shelf life of proteinaceous baits, which is only about one week. Traps baited with the new attractant caught 4-50 times fewer non-target insects than proteinaceous bait traps and caught 5-40 times fewer sterile males than trimedlure-baited traps. Overall, the new traps were 4.1 times more efficient in trapping Mediterranean fruit flies than protein-baited traps (IAEA, 1999). In addition, most of the females caught in the new traps were wild females, which increased the suppression effect of the control campaign. The sampling of live wild females also creates opportunities to assess the proportion of females having mated with sterile males (Vreysen, 2005).

## **4.2. Lepidoptera**

Moths are among the most devastating insect pests of fruits and vegetables and the following reports on the progress made in advancing the SIT with the codling moth, the cactus moth and the date moth.

### **4.2.1. The codling moth**

#### *Insect Quality Control and Management*

Adequate dispersal of released sterile insects is an important parameter for the efficient implementation of AW-IPM programmes with a SIT component. In the codling moth, flight behavior and dispersal are subject to genetic variation with about 10% of the genotypes encountered being mobile, and the remaining 90% can be classified as sedentary. Biological traits such as body weight, size and lifespan could be correlated with mobility (Schumacher et al., 1997). The implications of the selection for mobile versus sedentary type males in a rearing facility on the efficacy of a sterile male release programme, is an important factor that deserves further research.

The field performance of sterile male moths can be improved by raising them through diapause and by lowering the radiation dose from 250 Gy to 150 Gy (Bloem et al., 2004). Cost-effective methods to extract pupae from the diet, as a prerequisite for long distance shipment of pupae were conducted using techniques such as diet re-hydration, agitation, sieving, and pressurized water sprays. These procedures were followed by the application of a desilking agent to separate pupae from cocoons. (Carpenter et al., Proceedings of the Second FAO/IAEA Research Coordination Meeting, unpublished report).

Studies, to assess the mating compatibility of codling moth from different geographical locations were initiated with moths originating from the rearing facility of Osoyoos, British Columbia, Canada, and those native to South Africa (Blomefield et al., Proceedings of the Second FAO/IAEA Research Coordination Meeting, unpublished report). These crosses were expanded with codling moth material from Armenia, Argentina, Canada, New Zealand and Syria, after being shipped as diapausing larvae to the Entomology Unit of the FAO/IAEA's Agriculture and Biotechnology Laboratory in Seibersdorf, Austria. Preliminary results showed that there were no mating barriers between any of the populations tested (Gustavo Taret, unpublished report to the IAEA).

### *Genetics*

Significant progress has been made in elucidating codling moth genetics, as a basis for the development of genetic sexing strains. The karyotype of codling moths contains  $2n = 56$  chromosomes and the sex chromosome system is of the W-Z type, with females being WZ and males being ZZ (Fukova et al., 2005). Genomic *in situ* hybridization (GISH) and comparative genomic hybridization (CGH) have shown that the W and Z sex chromosomes can be easily distinguished from each other, making the codling moth a promising candidate for sex chromosome-based genetic sexing.

With codling moths, the W-Z sex chromosome system precludes the development of genetic sexing strains according to the same scheme used with the Mediterranean fruit fly (Franz, 2005). An innovative and elegant new scheme has therefore been proposed using conditional dominant lethal mutations that can be inserted into the female W chromosome by either transgenesis or translocation. The female moths carrying the transgene would be eliminated in the egg stage, and the non-transgenic males would be sterilized and released (Marec et al., 2005).

Transgenesis in the codling moth has been successfully carried out using the *piggyBac* transposon. Lines carrying the green fluorescent protein transgene were first produced over five years ago, and have since gone through 37 generations. This proves that the transgene has been stably incorporated into the codling moth genome. Preliminary studies have been carried out on the temperature sensitivity of a truncated form of the *Notch* gene, N60G11, in transgenic codling moths, indicating that this dominant temperature sensitive lethal gene could be used to develop genetic sexing lines of the codling moth (Neven et al., Proceedings of the Second FAO/IAEA Research Coordination Meeting, unpublished report).

Further research on the sex chromosomes of the codling moth is needed to elucidate translocation events and to localize transgenes on the chromosome. The optimal gamma radiation dose for inducing and isolating translocations between the Z and W chromosomes is 20 to 30 Gy. T(W:Z) translocations can

be detected by the appearance of a sex chromatin body in female polyploid nuclei. Twenty lines with suspected T(W:Z) translocations have been isolated (Makee and Tafesh, in press).

#### 4.2.2. The date moth

The date moth (*Ectomyelois ceratoniae* Zeller), also known as the carob moth, is a devastating pest of dates in Tunisia, Morocco, Algeria, Libya, Iran, Iraq, Saudi Arabia and Israel. In Tunisia, the date moth causes staggering economic losses with infestation rates as high as 90% in pomegranates, especially in the south of the country, 75% in pistachios, and 20% in dates (Mediouni and Dhoubi, in press). The government has established a maximum infestation level of 5% for dates destined for the export market. Since several years, the use of organochlorine insecticides has been completely banned and date moth control currently relies exclusively on the following measures:

- physically protecting dates with nets and plastic bags;
- the spraying with *Bacillus thuringiensis* (*Bt*);
- improved orchard management, including the removal of fallen dates and the elimination of other host trees from date plantations and
- post-harvest treatment (fumigation) of dates with methyl bromide.

Each of these control measures has its limitations, which has prompted the search for effective, environmentally safe alternatives. Researchers in Tunisia have started evaluating the use of the SIT as part of an IPM approach as a promising approach to controlling the date moth. The isolated nature of most of the date palm plantations in the southern part of Tunisia, offer an ideal opportunity to apply area-wide control efforts.

Most of the recent advances in using SIT to control the date moth have been improvements in the rearing. Efficient cylindrical oviposition cages with walls consisting of removable paper sheets were designed for reproduction of the moths. The cages are slowly rotated on rails to ensure even light distribution. The system is highly efficient as shown by the excellent fecundity of the female moths and the random distribution of the eggs on the paper sheets (Mediouni and Dhoubi, in press). A major breakthrough in the rearing of date moth was the development of an efficient artificial diet based on wheat bran, sucrose, glycerine and water. This development allowed the shift from individually reared moths on dates to the mass-rearing of larvae. The larvae are placed in trays stacked on trolleys, each holding 85 trays. Each trolley can produce between 160,000 and 170,000 adult moths (Mediouni and Dhoubi, in press).

An elegant system has been developed to automatically collect adult moths. This system is based on the one used at the codling moth production

facility in Canada. The pupae are kept on trolleys in a completely dark, climate controlled room. After they emerge, the adult moths are attracted to a light source and sucked through a duct into a cold room, where they are immobilized. This system significantly reduces labor costs, eliminates weak moths, and ensures that the moths selected for release are of high quality.

#### 4.2.3. The cactus moth

The cactus moth (*Cactoblastic cactorum* (Berg)), a native to Argentina, was first detected in Florida in the USA in 1989 (Habeck and Bennet, 1990). In Australia and the Caribbean, the cactus moth has successfully been used to control invasive prickly pear cacti (*Opuntia* spp.).

However, the accidental introduction of the cactus moth into the USA raised serious concerns for its potential spread to the *Opuntia*-rich areas of the western USA and Mexico (Hight et al., 2002). The range of the cactus moth in the United States is expanding at the alarming rate of 50 to 75 kilometers a year (Hight et al., 2002). At this rate, it is expected to reach Texas in 2007.

The spread of the cactus moth in North America threatens:

- the unique floral diversity of the deserts in the United States and Mexico (Perez-Sandi, 2001);
- the ornamental and landscape industry in the western United States (Irish, 2001);
- commercial plantations throughout Mexico, on which *Opuntia* is grown for food and cattle fodder (Soberon et al., 2001);
- local and international fruit markets;
- the food processing industry;
- the cosmetics industry, which uses *Opuntia* to make a wide range of products such as soaps and body creams; and
- the chemical industry, which uses *Opuntia* to feed insects used in the production of dyes.

Furthermore, *Opuntia* has long been used by local populations in regional cuisine, in traditional medicine, as a source of shade, and in constructing fences (Vigueras and Portillo, 2001).

No satisfactory method to control the cactus moth has so far been identified. Widespread insecticide use is not recommended because many of the *Opuntia* stands which need to be protected are located in ecologically sensitive areas (Leibee and Osborne, 2001). Injecting systemic insecticides into cactus stems is ineffective and costly (Pretorius et al., 1986). Neither pathogens nor natural enemies hold much promise because they are not very selective (Pemberton and Cordo, 2001). It has therefore been proposed to use the concept of F<sub>1</sub> sterility to study and to help contain the pest. Sterile F<sub>1</sub>

moths can be used in studies which cannot be safely carried out with fertile insects, including studies on:

- which native *Opuntia* species are potential hosts for the cactus moth;
- the geographical spread and the potential range of the cactus moth; and
- the potential impact of native natural enemies on the expansion of the cactus moth (Carpenter et al., 2001).

Furthermore, sterile F<sub>1</sub> cactus moths can be used in control programs to:

- eradicate the cactus moth in ecologically sensitive areas and in areas into which it may be accidentally introduced in the future;
- establish a barrier of sterile moths to prevent the moth from spreading; and
- provide for hosts for naturally occurring and introduced natural enemies in order to enable them to become established in the target area (Carpenter et al., 2001).

Research so far has focused on:

- determining radiation sensitivity in male and female moths;
- developing monitoring tools, including improved traps;
- elaborating techniques to estimate the population density of adult moths (Hight et al., 2003);
- using sterile female moths in traps for surveys (Bloem et al., 2003);
- identifying the female pheromone, which is now commercially available and is being evaluated in field studies; and
- developing an artificial diet for the mass production of cactus moth larvae.

## **5. ECONOMIC BENEFITS**

### **5.1. Fruit fly programs**

The integration of SIT in AW-IPM strategies to control fruit flies has resulted in enormous economic benefits. This is especially true for eradication programs, for which the economic impact can be directly measured (Hendrichs, 2000). The benefit-to-cost ratios reported have been very encouraging, and have been calculated to be:

- 146 for the California Mediterranean Fruit Fly Preventive Release Program, which protects the horticultural industry in California;
- 150 for the Mediterranean Fruit Fly Containment Program in Guatemala and Mexico, which protects the horticultural industry in Mexico and the United States; and



- 400 for the Mediterranean Fruit Fly Eradication Program in Chile, which opened export of horticultural products (Enkerlin, 2005).

SIT-based suppression programs have also resulted in substantial economic benefits. For example, in the Hex River Valley of South Africa, using pesticides to control fruit flies on table grape plantations used to cost US\$ 350,000 a year. Using SIT has reduced the cost to US\$ 130,000 a year. Furthermore, the number of crates which were rejected for export because of fruit flies has been reduced by 50%. The combined savings add up to US\$ 370,000, which is a significant amount considering that the actual grape producing area is not very large (Enkerlin, 2005).

## 5.2. Moth programs

Few data are available on the potential benefits of AW-IPM programs with an SIT component designed to control lepidopteran pests. In one study, the economic impact of a program to eliminate the codling moth from Syria was assessed. The program would use SIT in combination with pesticides over a period of fifteen years. Projected figures based on both direct and indirect benefits included a benefit-to-cost ratio of 3.98, an internal rate of return of 42%, and a return on equity of 5.9 (Walther Enkerlin, unpublished IAEA report).

## 6. PRIVATIZATION

The increased interest expressed by the private sector to produce and market sterile insects in the last years is an interesting and very encouraging trend. For example, companies which have recently started to commercially produce and market Mediterranean fruit flies include SIT Africa, Ltd. in South Africa and Bio-Fly in Israel.

The idea of privatizing the rearing and commercialization of sterile insects is not new, as is exemplified by the “De Groene Vlieg”, a Dutch private company that has produced sterile onion flies (*Delia antiqua* Meigen) since 1981 (Loosjes, 2000). The production facility can produce 400 million onion fly pupae per year, which is enough to treat 2,600 hectares, or 16% of the total Dutch onion crop.

Despite initial problems due to the growers’ lack of confidence in the technique, their misconception of the inverse-density dependence of the SIT and their over-confidence in chemical control, the programme has been very successful. Since 1998, the SIT program has been profitable, and the hectareage covered by the program has been increasing by about 5% a year. De Groene Vlieg has recently invested in increasing the capacity of their production facility.

### **SIT Africa, Ltd.**

In the Hex Valley of South Africa, a pilot AW-IPM program with an SIT component was carried out from 1999 to 2003. The aim was to suppress the Mediterranean fruit fly population on 10,000 hectares of table grapes. Cost-effectiveness was a primary consideration. The pilot program was financed primarily by growers' organizations, with limited funding from the government (Barnes et al., 2004).

A formal SIT Partnership was formed between the Deciduous Fruit Producers' Trust and the Agricultural Research Council, a para-governmental organization. The SIT Partnership covered the production costs of the sterile flies. Growers purchased sterile flies at a subsidized rate, and financed all field operations including the release of sterile flies. The Agricultural Research Council (ARC Infruitec-Nietvoorbij) managed the program supported by limited and irregular grants from the Western Cape Department of Agriculture. However, the SIT Partnership was not able to financially sustain the project. Investment capital was also in short supply because of unfavourable economic conditions. Therefore, privatization was considered as a promising alternative. In 2003, a private company, SIT Africa, Ltd., was established to produce and distribute sterile male fruit flies. Since 2004, the full cost of the SIT component has been covered by the growers, who purchase the sterile flies from SIT Africa. Furthermore, area coordinators contracted by SIT Africa provide expert technical assistance during release and monitoring operations (Barnes, in press).

### **Bio-Bee/Bio Fly**

Operations to suppress the Mediterranean fruit fly in the Arava/Araba Valley between Israel and Jordan) began in 1998. Until changes in airfreight regulations after September 11, 2001, the program relied on weekly imports of 15 million sterile male pupae from the El Pino Moscard facility in Guatemala. After the changes, shipment time was significantly lengthened, which increased mortality and reduced the quality of the sterile flies. This highlighted the need for the establishment of a Mediterranean fruit fly rearing facility in the region.

In 2003, Bio-Bee, an Israeli company that mass-produces and markets beneficial insects and mites for agricultural purposes (e.g. natural enemies for biological pest control and bumblebees for natural pollination in greenhouses and open field crops), conducted an economic feasibility study to establish a rearing facility. A new company, Bio-Fly, was established on a kibbutz in Sde Eliyahu. In March, 2005, Bio-Fly opened a production facility with a capacity of 20 million sterile male flies a week, which delivered its first shipment of flies to the Arava program in May 2005. Bio-Fly intends to expand the production facility in the near future because of increasing market demand.

## 7. FUTURE PROSPECTS

The trend towards agriculture that results in less pollution and lower levels of pesticide residues will increase the demand for less aggressive pest control tactics (Robinson and Hendrichs, 2005). These tactics should however not only be friendly to the environment but also be effective. In that respect, AW-IPM with an SIT component will certainly play an important role in the fight against a selected number of horticultural pests such as fruit flies and the codling moth. Unfortunately, AW-IPM has received little attention in Europe. Increased efforts are therefore needed to better inform both the scientific and political communities of the potential, the sustainability and the economic benefits of AW-IPM. However, it also needs to be emphasized that AW-IPM programs are complex and management intensive and require a high level of coordination between growers' organizations and independent management bodies in order to ensure that key control operations are correctly scheduled and executed. Consequently, success cannot be guaranteed and depends on a wide range of prerequisites (Vreysen et al., in press).

The recent involvement of the expanding private sector that already markets beneficial insects is therefore of particular interest and this trend can only be encouraged. These companies already have considerable expertise and experience in mass producing, shipping and handling beneficial insects, and can play a vital role in the improvement of several aspects of the SIT package (Robinson and Hendrichs, 2005).

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# TECHNIKA STERYLIZACJI SZKODLIWYCH GATUNKÓW OWADÓW JAKO ELEMENT ZRÓWNOWAŻONEGO, INTEGROWANEGO ZWALCZANIA SZKODNIKÓW UPRAW OGRODNICZYCH

Marc J.B. Vreysen, Jorge Hendrichs  
i Walther R. Enkerlin

## S T R E S Z C Z E N I E

Nieracjonalne stosowanie środków ochrony roślin o szerokim spektrum działania stwarza wiele niebezpieczeństw, a przede wszystkim kształtowanie się odporności agrofagów na te środki, pozostałości tych środków w płodach rolnych, skażenie środowiska naturalnego, „generowanie” nowych gatunków szkodników oraz redukcję liczebności owadów pożytecznych. Wzrastająca świadomość opinii publicznej dotycząca tych problemów sprawia, że staje się koniecznością wdrażanie takich metod zwalczania szkodników, których stosowanie będzie efektywne i jednocześnie przyjazne dla środowiska naturalnego.

Technika sterylizacji szkodliwych gatunków owadów może być powszechnie stosowana jako element integrowanych systemów zwalczania najważniejszych szkodników upraw rolniczych. Niniejsza publikacja jest przeglądem ostatnich osiągnięć w tej metodzie zwalczania szkodników. Przedstawia również praktyczne przykłady, w jaki sposób metoda ta powinna być stosowana.

**Słowa kluczowe:** technika sterylizacji owadów, zwalczanie integrowane