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Birth Control for Insects: The Sterile Insect Technique (SIT) for Controlling Fruit Fly (Tephritidae: Diptera) by Releasing Sterile Males

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Abstract

The aim of this technical brief is to summarize the results obtained from the laboratory and field trials conducted on birth control for insects in minimizing the impacts of fruit flies on horticulture. Several species of fruit flies belonging to Tephritidae family have a greater impact on global agriculture specially horticulture trade than almost any other pest. The introduction of fruit fly pests in an area pose a major risk to horticulture in affected countries due to the larvae damage to plant tissues before their harvest. One efficient, cost-effective and biologically-based fruit fly's control technology in use of the Sterile Insect Technique (SIT) that unlike to chemical control tactics is friendly to the environment and does not pose any health concern. The sterile insect technique is widely used in integrated programs against tephritid fruit fly pests, and it involves the mass-rearing and subsequent sterilization of large numbers of male insects of the target pest. The sterilized male insects are then released repeatedly over the infested areas, where these mate with the fertile wild females that consequently produce no offspring. The wild pest population can be effectively suppressed if the sterile males outnumber the wild males. The sterile flies are good fliers and marked sterile males have been recovered up to 24 miles away from their release point, and these are very transient throughout their life. Furthermore, it is important that operational programs implementing the SIT need continuous routinely revising and updating of all protocols to ensure increased efficiency, while two-way feedback is essential between the mass rearing facility and field operations. Finally, it is necessary to point out that this series of studies also cover all the aspects of total quality management for fruit fly rearing, sterilization, shipment, holding, and release as it relates to the SIT application to support rapid progress. A variety of studies have developed protocols to assess strain compatibility and to improve colonization procedures and strain management. Specific studies have also addressed issues related to insect nutrition, irradiation protocols, field dispersal and survival, field cage behavior assessments, and enhancement of mating competitiveness. In special situations of isolation, if the pest population is treated systematically on an area-wide basis with sterile males, complete eradication of pest flies can be achieved as sterile males can invariably seek out and mate with any remaining females of the target pest population, a feat that is difficult to achieve using insecticides.

Keywords

Sterile Insect Technique, SIT, Sterility, Sexual Behavior, Genetic Control

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1. Introduction

Over the last few decades, the colorful insect fruit flies of the dipteran family Tephritidae, are considered one of the most destructive and important pests of edible fruits and vegetables. Worldwide, fruit flies are well established in most of regions and cause a considerable severe damage to a wide range of fruits in many host species (Copeland et al., 2002; Sarwar et al., 2013; 2014 a; 2014 b; 2014 c; 2015; Shah et al., 2014). Fruit flies feed and breed on a variety of fruit and

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vegetable crops and are recognized as one of the key biosecurity pests threatening horticulture. The Sterile Insect Technique (SIT) is an environmentally benign approach for suppressing or eradicating insect pests, and it is widely used in integrated programs against tephritid fruit fly pests, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Hendrichs et al., 2002). The sterile insect technique is a promising, environmental friendly methodology for control or eradication of insect pests. The effectiveness of SIT depends on the quality and the ability of sterile laboratory males to search for females, mating compatibility and effective competitiveness with wild males (Orozco et al., 2007). In addition, the SIT is species-specific and is also suited to help eliminate outbreaks of invasive, newly introduced pest populations before these spread and become fully established. As such, it has no negative impact on natural enemies and pollinators, meeting the increasing public demand for safe and biologically responsive pest control option (Sarwar, 2015 a; 2015 b; 2015 c; 2015 d; 2015 e; 2015 f; 2015 g).

2. Control Strategies and Sit Applications

The SIT is effectively used as part of an integrated approach and in emergency situations is also effective to eliminate outbreaks of invasive pests. With assistance of the joint international programs, the SIT has been used successfully to suppress, contain, prevent establishment, or even to eradicate the fruit fly pests. This new area-wide integrated pest management (AW-IPM) SIT program is not only a preventative control option but is intended to have a positive impact on society by improving the quality of horticultural products at a lower cost, while protecting the environment and human health.

3. Controlling Fruit Fly Pest by Releasing Sterile Male Insect

Simply, SIT is birth control for insects, and it is used primarily to control or eradicate insect pests, usually crop pests or human and animal pests. The target insect is reared in great numbers, and the males are sterilized, usually using gamma radiation. The sterile males are released weekly in high numbers in the target areas, usually throughout the year. These mate with the wild females which results in infertile eggs being laid, provided that certain other population management activities are properly carried out, and the wild population then declines rapidly. It is harmless and ecologically approachable technique and can be used in orchards, urban and environmentally sensitive areas, and where conventional chemical treatment is not possible or is too intrusive from entire areas or countries. In addition, it has been applied to prevent incursions of the Mexican fruit fly (*Anastrepha ludens*), and to eradicate the Mexican fruit fly and the West Indian fruit fly (*A. obliqua*), and is also utilized against the South American fruit fly (*A. fraterculus*).

Through the application of the SIT component in AW-IPM programs, the melon fly (*Bactrocera cucurbitae*) has been eradicated, and the oriental fruit fly (*B. dorsalis*) and the guava fruit fly (*B. correcta*), are being suppressed in pilot areas to reduce losses in mango. In the Mediterranean region, the interest in the use of SIT against olive fruit fly (*B. oleae*) is growing. The recent introductions and spread of several Bactrocera species into other regions serve as a warning about the invasiveness of these exotic species. The SIT is proving to be one of the most successful insect pest control methods ever developed. At present, for controlling fruit flies, research and development, training, expert services and equipments are needed.

4. Sterile Insect Technique

Competition between sterile and wild males for females can end up with females mating with sterile male flies and therefore no offspring will be generated. The SIT aims at eradicating a species by flooding the population with sterilized males so that the chance of sterile males mating with wild females is greatly increased. The females generally mate once only under field conditions. The best example of success of this method is in eradication of melon fly from various Islands.

Prerequisites for this method are appropriate mass culture diets and facilities, capacity to produce hundreds or millions of flies per week and to monitor their fitness to compete with wild flies, appropriate techniques for sterilizing flies using Cobalt-60 or Cesium-137, effective transport and release techniques, and methods to evaluate the progress of the eradication program. These requirements mean a very expensive, sophisticated program and one that is appropriate for ecologically or geographically isolated areas into which wild flies are not likely to migrate and so dilute the effect of flooding the wild population with sterilized males. It is a technique that is not likely to be used in the field without significant financial justification. However, this species-specific technique is naturally pleasant and can provide effective control of fruit fly populations (International Plant Protection Convention. 2013). The SIT is effective only at low population levels of the target species and may be used for:-

i. Suppression, where SIT may be a stand-alone phytosanitary procedure or combined with other phytosanitary procedures to achieve and maintain low population levels.

ii. Containment, where SIT may be particularly effective in areas that are largely pest free (such as buffer zones) but that are subjected to regular pest entries from adjacent infested areas.

iii. Eradication, where SIT may be applied when population levels are low to eradicate the remaining population.

iv. Exclusion, where SIT may be applied in endangered areas that are subjected to high pest pressure from outside the area.

4.1. Sterile Fly Release

Sterile fruit flies can be released from the ground or from the air. Release intervals should be adjusted according to the longevity of the insect, but sterile flies are generally released once or twice per week. The frequency of release may be affected by circumstances such as pupae supply, staggered emergence and unfavorable weather. To establish sterile fly release density, it is important to consider the quality of the sterile fruit flies and the level of the wild population. After release of the sterile fruit flies, trapping and identification of the sterile and wild flies is important to evaluate the effectiveness of the release procedure. Moreover, released sterile flies are recaptured in the same traps that are used for detection of the wild population; this provides feedback on whether the desired sterile fruit fly density and sterile wild fly ratio is attained (International Plant Protection Convention. 2013).

4.1.1. Ground Sterile Fly Release

Ground release may be used when aerial release is neither cost-effective nor efficient (i.e., discontinuous distribution and relatively small area), or where additional releases are required to provide a higher density of fruit flies for a particular reason (e.g., in areas where a specified level of pest prevalence is exceeded). Adults for ground release are generally transported in containers or paper bags from the fruit fly emergence and release facilities to the release sites in cool conditions (less than 20 °C). Sterile flies may be released from predetermined release points under or in a tree canopy, preferably more than 100 m from any monitoring site, or these may be released from a moving vehicle.

4.1.2. Aerial Sterile Fly Release

Aerial release is more cost-effective than ground release for large-scale programs and it provides a more uniform sterile fruit fly distribution than ground release, which may clump sterile fruit flies in localized sites or along release routes. Once the release area is selected, it should be defined using Global Positioning System (GPS) and recorded in digitized maps using Geographic Information System (GIS) software, this will help to ensure the efficient distribution of sterile flies. The most common methods for aerial release are chilled adult and paper bag systems. The chilled adult release system is designed to handle large volumes of sterile fruit flies. The advantage of this system is that large numbers of fruit flies can be transported on each flight and uniformly dispensed into the environment. The paper bag release system is a relatively simple process whereby emerged flies within sealed bags are released when the bags are ripped open by hooks or knives located at the end of a chute exiting the aircraft. The operational programs use different methodologies to calculate release rates. To determine the release altitude, several factors need to be considered, including wind velocity, temperature, cloud cover, topography of the terrain, vegetation cover, and whether the area is an urban or a rural one. Release altitudes range from 200 to 600 m above ground level. However, lower release altitudes are preferred, especially in areas subjected to strong dominant wind currents (to prevent excessive sterile fruit fly or bag drift) and in areas where predation by birds is high and frequent. Release in the early morning, when wind and temperature are moderate, is preferable.

4.2. Sterile Fly Quality Control

Routine and periodic quality control tests are required to determine the effect of mass rearing, irradiation, handling, shipment duration, holding and releasing on the performance of the sterile flies according to desired quality parameters. Sterile male insects, mass-reared and released as part of sterile insect technique programs, must survive long enough in the field to mature sexually and compete effectively with wild males for wild females. Recognition of this problem and development of a corresponding quality control test could significantly improve the reliability and economics of sterile insect technology for fruit flies (Martha et al., 2007).

The study has assessed the performance of the mass-reared sterile *Anastrepha ludens* (Loew) flies interacting with wild populations from the different environments. Results show that the males of the wild populations differed in the time to the onset and peak of sexual activity. Nevertheless, the index of sexual isolation reflected the sexual compatibility between the wild populations and the mass-reared strain indicating that the sterile individuals mate satisfactorily with the wild populations. The male relative performance index showed that the sterile male is as effective in copulating as the wild males. The female relative performance index reflected a general tendency for wild females to copulate in greater proportion than the sterile females, except for the strains from two different environments. In general, the lower participation of the sterile females in copulation increases the possibilities of sterile males to mate with wild females. The relative sterility index showed that the acceptance by wild females of the sterile males (25-55%) is similar to that of wild males. Finally, the results suggest that sterile males successfully compete and are compatible with flies from different geographic origins (Orozco et al., 2007).

Compared to insecticidal control methods, SIT has some advantages including increased specificity and can be targeted to affected regions. The SIT programs in the past have failed due to continual immigration into the areas being targeted (Meats, 2003).

The most common method used to make fruit flies sterile for SIT programs is to irradiate them. The most effective time of the life cycle for irradiation to occur is when pupation is approximately 70% complete (Gilchrist and Crisafulli, 2005). The most effective irradiation dose rate for SIT programs should be at a level which makes individuals sterile without reducing their reproductive competitiveness. Studies have found that dose rate does not affect sterility induction, but higher does rates can cause stress mortality. The lowest practical dose rate should be used when irradiating for SIT control programs (Collins et al., 2008). Irradiated males are not reproductively disadvantaged against normal males in terms of female remating because levels of female remating have been found to be similar for irradiated and normal males (Harmer et al., 2006).

Although irradiation of male Q-flies causes changes in the temporal patterns of calling and courtship sounds, which could potentially affect mating competitiveness, there is no difference between the proportions of irradiated and untreated males which copulate successfully (Mankin et al., 2008). This is confirmed by Weldon (2005) who found that despite differences in behaviour, frequency of successful copulations and mating success are similar among wild, mass-reared and sterile males.

5. Distinguishing Sit Flies

Sterile male flies which are released into the wild should be marked, so if these are detected in a trap these can be distinguished from a wild infestation. One such way of marking flies is with fluorescent dust as they emerge from the pupae. When this happens, the ptilinum (a sac on the top of the head) is temporarily everted to break through the puparium, so the adult fly can emerge. Fluorescent dust adheres to the ptilinum while it is still everted, and after it retracts back into the head, some fluorescent particles are, in fact, embedded in the head, resulting in relatively permanent marking. This method of marking SIT flies, however, fails to mark a small number of sterile Q-flies. Strict international quarantine protocols to prevent the export of Q-fly to importing countries means that the detection of a very small number of flies that appear to be wild (not SIT released) could suspend international trade and have severe economic consequences to the agricultural sector. It is currently a very high priority to develop methods to unambiguously identify any captured Q-flies as wild or sterile. It should be noted that the cost of misclassifying sterile flies is very different to the cost of misclassifying wild flies. The only consequence of misclassifying a sterile Q-fly as a wild Q-fly is that one more fly needs to be examined using another method. However, if a wild fly is misclassified (as a sterile), then a wild population may remain undetected, with the possibility of larger subsequent outbreaks (Gilchrist and Crisafulli, 2005).

One method to unambiguously identify captured Q-flies as wild or sterile that is currently in development is using geometric morphometrics to analyze the subtle variation in wing shape between sterile and wild populations. Studies have shown that the results of this shape analysis can be used as the basis of a test to distinguish wild and sterile Q-flies. This method has a number of advantages over the fluorescent dust technique, it is very easy to perform, a lot faster to complete than other methods (several 100 wings/ day), uses mainly non-living tissue (the wing) so both live and long-dead specimens can be examined, and collecting data for wing shape analysis is relatively cheap, with the only requirements being a microscope-mounted video camera. Other methods which have been trialled include tests examining the difference in testes morphology (unreliable because some Q-fly bodies deteriorate while in traps for up to 2 weeks) and using DNA microsatellite markers to distinguish populations of wild and sterile Q-flies, which is very expensive and takes 5-10 days to complete (Gilchrist and Crisafulli, 2005).

6. Key Requirement of Sterile Insect Technique

Mattings between sterile males and wild females yield infertile eggs, thus reducing the reproductive potential of the wild population. To a large extent, the success of the SIT depends on the ability of released, sterile males to attract and obtain mattings with wild females. This capability is especially important as fruit fly SIT is the development of simple and inexpensive means to enhance the mating ability of sterile males. In recent years, several studies have focused on two modifications to the prerelease environment of adult sterile males that might enhance their performance in the field. One line of research has dealt with the composition of the adult diet, and in particular has examined whether supplementing the standard sugar agar diet with protein (yeast hydrolysate) enhances the mating frequency of sterile males. These studies have generated mixed results e.g., supplementary protein boosted the mating performance of sterile males in some studies (Kaspi and Yuval, 2000), but not in others (Shelly and McInnis, 2003). The satisfactory levels of compatibility and the good competitiveness of irradiated males and wild males may encourage the application of the SIT to control fruit flies population as stated below:-

i. Released sterile males compete with the wild males for mating with females. A high ratio of sterile males to wild males is therefore required. A key requirement of SIT is therefore that the sterile males be released into areas where the wild fruit fly population has been previously reduced to very low levels. If this is not done, there can be too many wild males mating with wild females, and the population may not decrease. Reduction of the wild fruit fly population to low levels before sterile releases commence is therefore essential.

ii. It is a proven technique, used in a number of other countries to create fruit fly-free areas, and can be very cost-effective.

iii. It is an area-wide and it must be applied over large areas e.g., 500 ha and larger.

iv. The SIT is highly environmentally friendly, making it very acceptable to export markets, which increasingly focus on ecologically compatible production techniques.

v. Depending on the size of the area under SIT and other factors, there are various options for release of sterile fruit flies, which can have a favorable impact on the cost-effectiveness of SIT.

vi. The SIT is management intensive, and it requires very good coordination and the cooperation of all the growers in an SIT area.

A variety of studies have been undertaken to develop protocols to assess strain compatibility and to improve colonization procedures and strain management. Specific studies addressed issues related to insect nutrition, irradiation protocols, field dispersal and survival, field cage behavior assessments, and enhancement of mating competitiveness (Carlos et al., 2007).

Unfortunately, the mass-rearing procedures inherent to the SIT often lead to a reduction in the mating ability of the released males. One potential solution involves the prerelease exposure of males to particular attractants. In particular, exposure of male Mediterranean fruit flies to ginger, *Zingiber officinale* Roscoe, root oil has been shown to increase mating success in laboratory and field cage trials. A field experiment compares the level of egg sterility observed in two coffee, *Coffea arabica* L., plots, with ginger oil exposed, sterile males released in one (treated) plot and non-exposed, sterile males released in the other (control) plot. Once per week in both plots over a 13-wk period, sterile males are released, trap captures are scored to estimate relative abundance of sterile and wild males, and coffee berries collected and dissected in the laboratory to estimate the incidence of un-hatched (sterile) eggs. Despite of that sterile wild male ratios are significantly lower in the treated plot than in the control plot, the incidence of sterile eggs has been significantly higher in the treated plot than in the control plot. Correspondingly, this study suggests that prerelease, ginger root oil exposure may represent a simple and inexpensive means to increase the effectiveness of fruit fly SIT programs (Shelly et al., 2007).

A study has estimated the sexual compatibility, mating performance and relative sterility between laboratory and wild flies of the Mediterranean fruit fly *C. capitata* by indices i.e., relative mating index, relative isolation index, isolation index, male relative performance index, female relative performance index, and relative sterility index. The results revealed that different doses of gamma radiation 10, 30, 50, 70 and 90 Gy have no effect on the various parameters of mating compatibility, performance and competitiveness of laboratory strain males of medflies when mated with wild males. Moreover, no significant assortative or dis-assortative mating has been observed. Therefore, it suggests that the laboratory strain males of medfly are compatible of mating with the wild males, at least employed under the laboratory conditions (Mahmoud, 2014). The study showed the wild flies may expose constantly to the natural environmental conditions, while laboratory flies may expose to fairly stable environmental conditions. This differences cause some changes in the behavior of laboratory flies. Attempts to obtain 100% sterility in males usually reduce quality, and it can often be better to reduce the dose so as to obtain a better induction of sterility in the field females by giving a more competitive male (Toledo et al., 2004; Dyck et al., 2005).

The sterile insect technique is a pest control technique with application in the integrated control of key pests, including the suppression or elimination of introduced populations and the exclusion of new introductions. It is an innovative fruit fly control program to protect fruit crops in establishing an Area Wide-Integrated Pest Management (AW-IPM) program that incorporates the sterile insect technique, to specifically target the breeding cycle of this major pest. The SIT is a method of biological control, where large numbers of sterile insects are released that compete with fertile insects to mate, which effectively reduces the overall population. Also, it is a naturally nonthreatening and cost-effective control option

(Sarwar, 2012; 2013; 2014 a; 2014 b).

7. Conclusion

In the present reading, there has been an observation on application of sterile insect technology, known as SIT that is a method in which sterile male fruit flies are released to mate with wild female fruit flies. It is safe and biologically responsive technique and pupae produced at the facility can be sold around the state for release where fruit fly is a major problem in horticulture production areas. This new facility can help to eradicate incursions of fly pests into an area and control flies in major horticulture production areas in the states, while maintaining leadership in biosecurity. Development of SIT to combat fruit fly is a further demonstration from strategic priority. Such a program is directly relevant to many fruit growing regions. In contrast, conventional control methods have a narrow focus in protecting crops from direct attack by pests. Chemical controls are increasingly coming under scrutiny due to environmental and health concerns and researchers have responded to the need to find alternate and softer in-field control options for flies by incorporating the SIT in an integrated program. The sterile insect technique is arguably the most ecologically-compatible means of pest control in existence time. It is not a stand-alone technology, but should be integrated with other pest management technologies, such as bait application and sanitation, in an area-wide program. Fruit fly compared with bisexual strains, and the poor mating capability of the released males severely limits their effectiveness. Realizing the full benefits of male-only releases therefore will require modification of current mass rearing procedures to improve the mating ability of released sterile males. The application of sterile insect technology can be used as a practical mitigation option for minimizing the global warming potential of horticulture ecosystem and enhancement of farm produces. The information presented in this paper might be helpful on developing and validating procedures to improve the overall quality of sterile fruit flies for use in area-wide integrated pest management (AW-IPM) programs with a sterile insect technique component.

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