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REVIEW ARTICLE

Sterile Insect Technique (SIT) as a component of area-wide integrated management of fruit flies: Status and scope

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ABSTRACT : The Sterile Insect Technique (SIT) is an effective, species specific and environment friendly approach to achieve area-wide management of insect pests and has been successfully applied against several species of fruit flies like *Ceratitis capitata* Wied, *Anastrepha ludens* Loew and *Bactrocera* spp. in different countries. It involves releasing a large number of specially reared and sterilized male insects into the target area where they mate with wild females of same species resulting in failure of off-spring production thus gradually bringing down the pest population.The SIT was conceived by Knipling in 1955 and was used to successfully eradicate the New World Screwworm, *Cochliomyia hominivorax* Coquerel, a cattle pest, from North and Central America.This can be used in a wide range of situations either for prevention of establishment of new pests or to suppress or eradicate the existing pests. Use of SIT resulted in an enormous growth in the export of fresh fruits and vegetables in Mexico, Chile, South Africa, USA etc. In this paper, we attempted a concise review of the mechanism, application, status and impact of SIT on area wide management of fruit flies across the world and the current Indian scenario of this technology.

Keywords: Area wide IPM, *Bactrocera* spp., fruit flies, radiation, sterile insect technique

INTRODUCTION

There are more than 400 species of fruit flies (Diptera: Tephritidae) known to infest several fruit and vegetable crops. Although, many insect pests attack fruits and vegetables, none had gained greater notoriety than Tephritid fruit flies and they are recognized worldwide as the most potential threat to the horticultural industry (Verghese *et al.*, 2002; Allwood and Drew, 1997; Barnes, *et al.*, 2004; Ekesi and Billah, 2007). Besides causing direct losses in yield and marketability, they are barriers to international trade of fresh fruits and vegetables. Unlike many other pests, the effective management of fruit flies cannot be achieved solely at farm level and the lapse at a particular orchard or farm could have wider implications for entire region or commodity (Verghese and Rashmi, 2014). Different traps and lures are being used over decades for the purpose of monitoring as well as mass trapping male flies. The first attractant for male fruit flies was methyl eugenol for *Bactrocera zonata*, discovered by Howlett in 1912 (Verghese*et al.*, 2013) immediately followed by kerosene for Mediterranean fruit fly, *Ceratitis capitata* Wied. in 1913 (IAEA, 2003). Subsequently, Angelica seed oil (Steiner *et al.,* 1957) and trimedlure (Beroza *et al.,* 1961) were also used to trap medflies. Beroza and Green (1963) demonstrated cuelure to be an effective attractant for *Bactrocera cucurbitae*. Food baits based on protein solutions, fermenting sugar solutions, fruit juices, and vinegar have been used since 1918 for the capture of females of several species (IAEA, 2003).

In India, the management strategies being followed for *Bactrocera dorsalis* on mango include crop sanitation, male annihilation technique (MAT) using methyl eugenol traps, bait splashes, chemical interventions and post-harvest measures like hot water treatment, vapor heat treatment (VHT) and irradiation (Verghese *et al.*, 2014). Of late MAT is being widely adapted by mango growers in India, thanks to the proactive role played by the organizations like ICAR-Indian Institute of Horticultural Research and some State Departments of Horticulture. However the lack of community level participation, unwillingness of contract farmers to erect traps and immigration of mated females from other fields not adapting the technology, are the major constraints in achieving desired results. Under such circumstances, having a strategy to address the problem on area-wide basis would be of great help in developing fruit fly free zones. Keeping in view the growing demand for residue free produce across the globe, it is widely believed and demonstrated that the sterile insect technique (SIT) is one such technology to fill the gap in the overall objective of area-wide management of fruit flies (Armstrong *et al.*, 1989; Jordan, 1993; Hansen and Johnson, 2007).

The area-wide pest management (AW-PM) is one of the most rational ways to control major agricultural insect pests (Klassen, 2003) and is defined as IPM against an entire pest population within a delimited geographic area, with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area (Dyck *et al.*, 2005). It requires long-term planning and coordinated efforts to successfully implement the strategy and is essential in circumstances where a particular region or zone has to be declared free from a target pest. Bringing down population of highly mobile pests on an area-wide basis is not only safe and effective but also more profitable, than on a farm-by-farm basis (Carlson and Wetzstein 1993). This approach involving SIT is highly relevant and desirable in case of fruit flies which are of quarantine importance.

SIT - WHAT AND HOW

The Sterile Insect Technique (SIT) means, in simple terms, insect birth control. According to the International Plant Protection Convention (FAO, 2005), the SIT is

Table 1.Countries where SIT is being integrated into area-wide fruit fly management

Country	Fruit fly species	Objective
Argentina	Mediterranean fruit fly or Medfly, *Ceratitiscapitata* Wiedemann	Eradication
Australia	Queensland fruit fly, *Bactroceratryoni* Froggatt	Prevention, Eradication
Brazil, Chile	Medfly, *Ceratitis capitata*	Prevention
Brazil	Medfly, *Ceratitis capitata*	Suppression
Guatemala	Medfly, *Ceratitis capitata*	Containment, Eradication
Israel	Medfly, *Ceratitis capitata*	Suppression, Eradication
Japan, Okinawa	Melon fly, *B. cucurbitae* Coquillett	Prevention
Jordan	Medfly, *Ceratitis capitata*	Suppression, Eradication
Mexico	Medfly, *Ceratitis capitata* Mexican fruit fly, *Anastrephaludens* Loew West Indian fruit fly, *A. oblique* Macquart	Eradication Prevention, Suppression Prevention, Suppression
Peru	Medfly, *Ceratitis capitata* South American fruit fly, *A. fraterculus* Wiedemann	Suppression, Eradication Suppression
Portugal, Madeira, South Africa, Spain, Tunisia	Medfly, *Ceratitis capitata*	Suppression
Philippines	Philippine fruit fly, *B. philippinensis* (Drew & Hancock)	Suppression
Thailand	Oriental fruit fly, *B. dorsalis* Hendel Guava fruit fly, *B. correcta* Bezzi	 Suppression
USA, California, Florida	Medfly, *Ceratitis capitata*	Prevention
USA, Hawaii	Melon fly, *B. cucurbitae*	Suppression
USA, Texas	Medfly, *Ceratitis capitata*	Suppression, Eradication

(Source: FAO, 2003)

defined as a method of pest control using area-wide inundative releases of sterile insects to reduce fertility of a field population of the same species. It involves releasing a large number of sterilized male insects into the environment where they mate with wild females leading to the failure of off-spring production by the latter. This concept was first proposed by Knipling in 1955 (Knipling, 1955) and successfully used to eradicate New World Screwworm, *Cochliomyia hominivorax* Coquerel, a cattle pest, from North and Central America (Klassen and Curtis, 2005). Even before Knipling, the idea of releasing genetically mutated insects to spread deleterious mutations into the wild population and thereby reducing it was put forward by Serebrovskii (Serebrovskii, 1940). Since then, SIT has been used to prevent, control and eradicate populations of agricultural and veterinary insect pests around the world.

Since its first use in 1950s, SIT has been successfully employed against several fruit flies. Notable among them are eradication of the melon fly, *Bactrocera cucurbitae* Coquillett from Japan (Kuba *et al.*, 1996) and the Queensland fruit fly, *Bactrocera tryoni* Froggatt from Western Australia (Sproule *et al.*, 1992). The preventive application of SIT was proved against the Mediterranean fruit fly, *C. capitata* in California and Florida, USA (Dowell *et al.*, 2000; Barry *et al.*, 2004), Mexico (Hendrichs *et al.*, 1983) and Chile (Gonzalez and Troncoso, 2007) (Table 1).

MERITS AND REQUIREMENTS

As per the International Standards for Phytosanitary Measures No. 3 of the International Plant Protection Convention, sterile insects are categorized as beneficial organisms and SIT is among the most environment-friendly insect pest control methods ever developed. Since sterile insects are not self-replicating, they cannot become established in the environment and unlike classical biological control, does not involve introducing non-native species into an ecosystem (http://www-naweb.iaea.org/nafa/ipc/sterile-insect-technique.html). The major advantages of SIT are that it is species-specific, no ill effects on the non target organisms, environment or on human health, and is compatible with organic agriculture as it does not involve any toxic chemicals. Another important merit of SIT is that the efficacy is inversely density dependent, unlike other pest control strategies (Wimmer, 2005; Hendrichs *et al.*, 2007).

For SIT to be successful, the following are essential requirements (Klassen, 2005).

* The target pest must be a good candidate for suppression by the area-wide integration of the SIT with other methods
* The target pest must be amenable for mass rearing
* The adult stage should not be a pest or vector
* Biology and Ecology of the target pest must be thoroughly understood
* There must be strong stakeholder cohesiveness, and community level commitment to the campaign
* Legal authority is required to execute all aspects of the programme

COMPONENTS OF SIT

The SIT involves industrial-scale mass production of insects and subsequent sterilization by gamma-radiation with a dose that induces sterility but does not significantly impair the other abilities of the sterile males such as flight, mating and transfer of sperm to wild females. The sterile insects are released on a sustained and area-wide basis to target the total pest population in the defined area and in sufficient numbers to achieve appropriate sterile-to-wild insect overflooding ratios. This leads to a reduction in the proportion of fertile mating in the wild population and results in its decline (Knipling, 1968). The success of the SIT relies on the ability of the released sterile males to transfer to the wild female's functional sperm that succeeds in fertilizing the eggs. The number of times a female mates is not important, providing that the sperm from the sterile male is competitive with the sperm from the wild male. As the wild population declines and the numbers of released insect remains constant, both the proportion of sterile matings and the rate of suppression increase.Hence the efficiency of SIT is inversely density dependent (Pereira *et al.*, 2013). Knipling (1968) recognized that the level of suppression required to stabilize the density of a population depends on its intrinsic rate of increase (Table 2). He estimated that an overwintering screwworm population typically increases approximately five-fold for the next two or three generations.

Mass Rearing of fruit flies for SIT

A crucial component in establishing a successful SIT program is an efficient and cost effective mass rearing system to produce high quality insects. Since the first insect mass-rearing facility was built in Florida for the New World screwworm fly in 1950s, the SIT has progressed for other pest insects from the laboratory

Table 2. Rates of mortality and survival required to maintain a stable pest population

Intrinsic rate of increase between generations	Number of progeny per female	Number (fraction) that must survive to prevent population from declining	To prevent population increase	
			Number (fraction) that must die	Percentage that must die
2-fold	4	2(1/2)	2 (1/2)	50
3- fold	6	2(1/3)	4 (2/3)	67
4-fold	8	2(1/4)	6 (3/4)	75
5-fold	10	2(1/5)	8 (4/5)	80
10-fold	20	2(1/10)	18 (9/10)	90
20-fold	40	2(1/20)	38 (19/20)	95

Source: Klassen, 2005 (used with permission)

bench to the large scale 'factory' level of sophistication. For instance the El Pino facility in Guatemala has the capacity to produce close to 3000 million sterile Mediterranean fruit flies per week. Other facilities around the world have also been built for different pest insects (http://www-ididas.iaea.org/IDIDAS/ default.htm). Production of a large number of flies, in the range of millions per week necessitates an artificial diet and a quality controlled rearing facility. Artificial diets must supply the flies with all the nutrients required for achieving maximum potential fecundity, longevity and mating success. Diets generally include a protein source, an energy source (normally carbohydrates), B complex vitamins, and mineral salts (Dadd, 1985). Kaur and Srivastava (1991) have demonstrated that diets without B complex vitamins, folic acid, or biotin will reduce fecundity and eclosion rates in fruit flies. In Brazil, autolysed yeast and yeast extract were used as substitutes to the expensive hydrolysed protein to rear *C. capitata* for SIT programme (Moreira da Silva Neto *et al.*, 2012). A larval diet for peach fruit fly, *B. zonata* consisting of sugarcane bagasse, ground maize, sugarcane sugar, waste brewer's yeast, wheat bran, benzoic acid, nipagin and water yielded desirable quality attributes of mass rearing i.e., 85% egg hatch, > 67% pupal recovery, > 4.2 g pupal weight, > 95% pupation, > 89% adult emergence and > 65% fliers (Sooker*et al.,* 2014).

Ideal dose and stage to induce male sterility

Sterility may be caused by the inability of males to produce sperm, or sperm inactivation or dominant lethal mutations in the reproductive cells of either the male or female (LaChance *et al.*, 1967). All of these mechanisms may be induced by exposing insects to gamma rays, X-rays, or certain chemicals (Bakri *et al.*, 2005). In addition, sterility may also be induced by insect growth regulators which can be transferred from a treated male to an untreated female during mating, subsequently disrupting the development of the embryo by interfering with endocrine mechanisms (Hargrove, 2005). However gamma radiation from the radioisotopes ^{60}Co and ^{137}Cs is the most commonly used radiation source for the SIT programmes. These isotopes have long half-lives and produce gamma rays with relatively high energy. Determining the optimum dose is a prerequisite to initiate SIT. The dose should be high enough to cause sterility without affecting the survival and mating ability. The dose required for sterilization varies with species of insect. For instance, the dose needed is less than 5 Gy for blaberid cockroaches while it is 300 Gy or more for some arctiid and pyralid moths. Within a species, insect age and stage during irradiation also influence the absorbed dose required for sterilization. For a given species of fruit fly, the optimum doses being followed in different countries seem to differ (Table 3). In case of *C. capitata*, the dose ranges from 90 Gy in South Africa to 145 Gy in Gautemala. The doses advocated for *Bactrocera* spp. are in the range of 64-90 Gy (Bakri *et al.*, 2005). The selection of the insect development stage and age is also critical for the success of sterile insect release programme. For many holometabolous species, pupal stage is ideal for irradiation. Fruit flies are usually irradiated one or two days prior to adult emergence (Fletcher and Giannakakis, 1973).

Table 3. Radiation doses used to induce sterility in different insect species in different countries

Country	Insect Targeted	Radiation Dose (Gy)
Argentina	*Ceratitis capitata*	110
Canada	*Cydia pomonella*	150
Chile	*Ceratitis capitata*	120
Guatemala	*Ceratiti scapitata*	100-145
Mexico	*Anastrepha ludens, A.obliqua* *Ceratitis capitata*	80 100
Mexico	*Cochliomyia hominivorax*	80
Philippines	*Bactrocera philippinensis*	64-104
Portugal	*Ceratitis capitata*	100
South Africa	*Ceratitis capitata*	90
Thailand	*Bactrocera dorsalis*	90
USA (Hawaii) CDFA/USDA	*Ceratitis capitata*	140
USA (Hawaii) ARS/USDA	*Ceratitis capitata*	120
USA (Texas)	*Anastrepha ludens*	70

(Adapted with permission from Bakri *et al.*, 2005)

Assessing Release Densities

Since production of sterile insects is a cost intensive operation, it is essential to have a system to assess the requirement so that releases can be made in a rational way. The number of sterile males to be released depends on the existing density of wild flies in the target area and also the objective of the SIT programme (Table 4). The ratio of sterile to wild males is low (50:1) where the objective is prevention while it is almost three times higher where eradication is aimed at. The density of wild males can be roughly estimated based on the number of males trapped per trap per day as described in IAEA (2003). However, Itô and Yamamura (2005) developed a methodology to determine population density with more accuracy.

Table 4. Requirement of sterile male to wild ratios for different objectives of SIT

Objective	Sterile: Wild ratio
Suppression	100:1
Eradication	150:1
Containment	150:1
Preventive Release	50:1

(Source: FAO/IAEA, 2016)

RECENT ADVANCES IN SIT

Rearing techniques

Rearing of the insects for themass release is an important component of the SIT. Therefore advancement in the rearing techniques of the fruit flies with simple and cost effective procedures will be beneficial. “Filter rearing system” has been devised to maintain the genetic integrity of genetic sexing strains of the Mediterranean fruit fly (Fisher and Cáceres, 2000; Parker, 2005). Transgenic strains have been produced in *Drosophila* that induces 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 achieved by the addition of antibiotics to the larval diet that shuts down the lethal gene (Heinrich and Scott, 2000; Thomas *et al.*, 2000) and in the medfly, similar strains have been produced (Gong, *et al.*, 2005). However, these strains will require further refinement as they are not completely sterile in matings with wild females, and the majority of the lethality occurs in the late larval stage. Addition of nutritional supplements mainly proteins are crucial for sexual development and signalling in some species, and often significantly increase competitiveness (Shelly *et al.* 2002; 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).

Genetic sexing of strains

Even though highly successful area-wide SIT programmes have been conducted by the simultaneous release of irradiated males and females (Lindquist *et al.*, 1992; Wyss, 2000), for many agricultural pests it would be highly preferable to eliminate females from the release population. The seperation of females prior to large-scale sterile insect releases is of great importance, both in terms of economics of production and biological efficiency in the field (Franz, 2005; Parker, 2005). In the Mediterranean fruit fly, where genetic sexing strains are in widespread use, a mulit-fold increase in field efficiency was observed (Rendón *et al.*, 2004; Cáceres, 2002). Application of the molecular approaches for genetic sexing would either lead to death of the females or transformation of females to males (Lagos *et al.*, 2007). In *Drosophila*, a tetracycline repression system has been used to construct female killing systems (Heinrich and Scott, 2000, Thomas *et al.*, 2000). 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; Hediger *et al.*, 2010).

Marking

Sterile insects for release are usually marked with a fluorescent powder to recognize them from wild insects. Genes encoding fluorescent protein markers, *i.e.* green fluorescent protein (GFP) and red fluorescent protein (DsRed) can now be introduced into fruit fly pests to provide a more secure and easier system for identifying sterile insects (Morrison *et al.,* 2010).

Transgenic strains

Transgenic strains have been created in *Drosophila* that instigates embryonic lethality in eggs fertilized by released fertile transgenic males carrying a dominant lethal gene. By the incorporation of antibiotics to the larval diet will shut down the lethal gene (Heinrich and Scott, 2000; Thomas *et al.*, 2000). This methodology is used in the medfly to produce similar strains (Gong *et al.,*2005).

Advances in releasing and distribution of sterile insects

Several advances have taken place in the mode of release of sterile flies. Sacks and boxes are replaced with chilled containers, and a precise calculated release into the environment to accomplish the required number per unit zone (Dowell *et al.*, 2000). Computer software linked to a satellite-guided aerial navigation system is programmed to deliver an adjustable number of sterile insects 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 analyzed after each flight (Tween, 2005).To increase the accuracy and ease with which insect populations can be monitored before, during, and after the implementation of a programme, GPS and GIS are being used. Improved aircraft navigation systems also facilitate the accuracy of the sterile fly releases. This technology serves in accurate navigation and geo-referencing the area of insect traps, hosts and other information has greatly reduced the costs (Tween, 2004).

ECONOMIC IMPACT OF FRUIT FLY AW-IPM WITH SIT

The benefits accrued from SIT to the growers, society and nation include the production of more and better-quality horticultural products at a lower cost which increases the food supply, diversifies markets and creates new jobs at the same time without affecting the environment. Nevertheless quantifying the impact of AW-IPM programmes, that use SIT to control fruit fly pests is complex as they affect practically the whole horticultural food chain (Enkerlin, 2005).

Several workers and agencies attempted to assess the economic impact of using SIT in fruit fly management in different countries (Reyes *et al.* 1991; USDA/APHIS, 1993; Mumford *et al.,* 2001; Kakazu, 2002). The ultimate indicator used was the benefit cost ratio after taking into account the increase in production, exports and decline in cost of plant protection. It was estimated that the benefit cost ratio was 150 in Mexico and US after implementing the Mediterranean Fruit Fly Containment Programme (Programa Moscamed) while it was as high as 1600 and 1900 in Chile and South California respectively where control and prevention of the same pest were achieved through SIT proramme

(Lindquist and Enkerlin, 2000). In Japan, Okinawa Island was made free from melon fly thus helping to significantly enhancing export of horticultural products (Kakazu, 2002). The high economic returns are possible mainly because of the environment-friendly and area-wide nature of the SIT technology. This technology allows cost-effective suppression and containment. The AW-IPM with SIT facilitates protection of high-value horticulture industries at a relatively low cost as demonstrated in Argentina, Australia, Chile, Mexico, and the USA (Enkerlin 2003). In contrast, the worldwide benefit/cost ratio of insecticides has been estimated at 4:1, if indirect costs are excluded, and only a 2:1 ratio if indirect environmental and public health costs are included (Pimentel, 1991).

STATUS OF SIT IN INDIA

In India, during 1973, SIT was used to control mosquitoes. For the control of *Culexquinque fasciatus* in Delhi, 300,000 sterile males were released daily over 14 weeks. Sterilization was carried out with cytoplasmic incompatibility and chromosome translocation (Curtis *et al.*, 1982). Whereas Yasuno *et al.* (1978) released 38 million sterile males over 25 weeks period using chemoterilization with thiotepa. At Bhabha Atomic Research Centre (BARC), Mumbai, attempts have been made to apply SIT for controlling red palm weevil, *Rhynchophorus ferrugineus* Oliv., potato tuber moth, *Phthorimaeao perculella* Zeller and spotted cotton bollworm, *Earias vittella* Fabricius in collaboration with some State Agricultural Universities (DAE/BARC, 2016). Prabhu *et al.* (2010) found irradiating one day old adults of red palm weevil at 15 Gy resulted in 100 % sterility and irradiation had no effect on the attraction to pheromone lures. Pilot studies on using SIT against red palm weevil in coconut were also conducted by Kishnakumar and Maheswari (2007). In case of fruit flies, a pilot study was initiated at ICAR-IIHR, Bengaluru in collaboration with the BARC to evaluate the feasibility and efficacy of SIT under the existing agro-climatic situations for management of *B. dorsalis* and *B. cucurbitae*. The dose of radiation for both the species to induce sterility was optimised and an artificial diet for laboratory rearing was developed (Reddy *et al.*, 2016).

SUMMARY

With the increasing demand for safer and residue free agricultural produce, the search for newer and environmentally benign means of pest management has been intensified. Area-wide pest management involving SIT is a rational approach to manage pests like fruit flies which drastically affect the horticultural industry of several tropical countries including India. Though conceived and demonstrated as early as the 1950s, and been successfully used in several countries, SIT is yet to make a mark in India, where mango exports are limited mainly because of the quarantine restrictions related to the Oriental fruit fly, *B. dorsalis*. There is a scope to apply SIT to create few fruit fly free zones which in turn would help boosting marketable yield and international trade. Voluminous information is available on the AW-IPM of fruit flies involving SIT, thanks to the efforts and contribution of FAO/IAEA team of scientists. We attempted this concise review with a major objective of sensitizing the researchers and other stake holders in India to explore the possibility of integrating this safe technology in devising the sustainable fruit fly management programmes. As rightly stated by Bakri *et al.* (2005), in the coming days, the SIT will contribute even more to improved food security worldwide by increasing fruit and vegetable production in a cost-effective, environmentally clean, and sustainable manner.

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