

Sterile Insect Technique: international framework to facilitate transboundary shipments of sterile insects

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Summary

The sterile insect technique (SIT), as part of an integrated pest management (IPM) approach, has been successfully applied since the 1950s in large-scale programmes to prevent, contain, suppress, and eradicate key insect pests in many countries worldwide. During this period, over 1 trillion live sterile insects have been shipped across borders. The very few incidents from this significant trade were managed and resulted in no significant impacts. The phyto and zoosanitary regulations in place and required so far by importing countries have been simple and have facilitated transboundary shipment of sterile insects. This has been done mostly under the framework of cooperative agreements between governments of involved countries and under technical cooperation projects of the United Nations. The shipment of sterile insects from other sources outside this governmental framework, including public-private facilities, however, has been complicated, despite the availability of harmonised international guidelines in some cases, such as for fruit flies. There is a great potential use of the SIT for control of endemic pests or against the growing threat of invasive pests that can affect whole regions and continents. Since SIT is species-specific with negligible risk of introducing unwanted invasive species to the environment and with the advantage of reducing insecticide use, a harmonised framework that recognises the low risk of SIT, would facilitate shipments of sterile insects across borders and help

to expand the use of this effective and environment friendly technology. The scope of this chapter is limited to insects that have been sterilised using ionising radiation.

Keywords

Harmonised guidelines – Ionising radiation – Sterile insects – Transboundary shipments.

Introduction

The sterile insect technique (SIT) has been successfully applied in large-scale operational programmes to prevent, contain, suppress, and eradicate pests in many countries worldwide (1). The SIT is species-specific and, as such, it enables pest control without introducing potentially invasive insect species into ecosystems with threats to biodiversity. Unlike insecticides and other control methods, it acts in an inversely density-dependent manner and as a result increases its efficiency with decreasing population density (2).

The SIT, as described in this article, specifically uses ionising radiation (Gamma, X-rays, or e-beam) to sterilise mass-reared insects so that they cannot produce offspring when released in the field and mating with wild females. As a result of the physical action of radiation, chromosomes break in the germinative cells of the insect's sperms and ovules, causing lethal dominant mutations that prevent the development of the embryos (3). This results in the introduction of sterility in the wild population and the reduction of a pest population over time.

The SIT relies on the sustained and area-wide release of large numbers of sterile insects over areas infested with target pests. Prior to their release, sterile insects are mass-reared, sterilised, packed, shipped, emerged as adults from the puparia, fed and matured, and then loaded into delivery vehicles for aerial or ground releases (Fig. 1). The conditions under which these activities are conducted are as relevant to the overall success of – and negligible risk from – SIT activities as is the production of high-quality sterile insects (4).

[Place Figure 1. here]

Sterile insects have been used for pest prevention, such as the preventative release programme (PRP) in California, USA, where sterile Mediterranean fruit flies are released weekly by air over large areas at risk from introductions of this invasive pest (5). They have also been used in biological containment barriers, such as the Mediterranean fruit fly barrier located along the Mexico-Guatemala border, where over one billion sterile Mediterranean fruit flies are released per week to prevent spread to the pest free areas north of the barrier (6). For suppression, such as the codling moth programme (OKSIR) in Okanagan, British Columbia, Canada, sterile moths are continuously released over apple orchards to control the pest as a replacement of synthetic insecticides (7). For eradication, sterile New World Screwworm were reared and released throughout the whole of Central America (8). SIT has also been used to eradicate tsetse fly (*Glossina austeni*) populations on Unguja Island, Zanzibar (1994–1997). It was the first SIT-based eradication effort against a tsetse species (9). An effort to eradicate *Glossina palpalis gambiensis* in Senegal is ongoing (10). SIT is also used to eradicate incipient outbreaks of invasive pest species, such as the cactus moth (*Cactoblastis cactorum*) in the Yucatan Peninsula, Mexico (11), a major outbreak of the New World Screwworm (*Cochliomyia hominivorax*) in Libya (12) and the Mediterranean fruit fly (*Ceratitidis capitata*) in the Dominican Republic (13).

Benefits and risks of Sterile Insect Technique

The return on investment of past and current SIT programmes is unquestionable, with benefit to cost ratios ranging from US\$ 2.80 for each dollar invested in the case of a Mediterranean fruit fly population suppression programme in South Africa, to as high as 1,000 to 1 in the case of a Mediterranean fruit fly prevention programme in Chile (14). The SIT has been applied for over 65 years against several insects which are key plant pests, such as fruit flies and moths, and key livestock pests, some of which are vectors of zoonotic diseases including new world screwworm (NWS) and tsetse flies. More recently, SIT has been developed against *Aedes aegypti* and *Aedes albopictus* mosquitoes and vectors of human disease such as dengue, chikungunya

and Zika. Pilot trials are under way in several countries, including Brazil, People's Republic of China, Cuba, Germany, Italy, Mauritius, Mexico, Singapore, Spain, and the United States of America (USA). Through the pilot trials the impact of releases of sterile male mosquitoes over the wild populations and the reduction in the transmission and incidence of disease in the human population has been shown (15).

Sterile insects used in SIT programmes have been shipped across borders to many countries successfully since 1963. In the 58 years of transboundary shipments, although not well documented, it is known in the industry that very few incidents have occurred. Some incidents related to production protocols, demonstrating the need for quality assurance in the sterilisation process prior to shipping and highlighting the advantages of well-resourced, centralised production facilities. For example, one case was an error in irradiation of New World Screwworm (NWS) that resulted in the release of fertile flies. In that instance the releases were near the production facility and the fertile flies were contained and eliminated in a short period of time with no significant impacts on livestock and human health. Another case was the escape of fertile Mediterranean fruit flies (or Medfly) from a production unit. The flies were detected through a trapping network and contained around the facility by applying a rapid response protocol with no further consequences. Another issue has arisen due to delay in international transport, resulting in the inefficient release of flies with poor quality. Labelled boxes with sterile Medfly pupae were held at Customs during transit causing an excessive period in anoxia before arrival to the destination. This was due to inadequate documentation causing confusion at Customs.

Production and sterilisation processes

Proper production conditions are key to virtually eliminating the risk from the large shipments of sterile insects. Production of sterile insects for use in SIT programmes involves mass rearing at high standards. Mass rearing facilities employ standard operating procedures or manuals that describe the production process in detail. The process involves breeding colonies, egg seeding, larvae rearing, pupae rearing,

adult rearing and irradiation. Generally, each of these steps requires separate rearing areas and specific climatic conditions including temperature and relative humidity. The diet used to feed adults in the breeding colonies and the one used to rear the larvae are specific to each insect species. During the rearing of the different biological stages of the insect, diets are kept free of pathogenic microorganisms by manipulating the pH, by applying specific antibiotics and fungicides and in certain cases through sterilisation. To maintain high levels of hygiene inside the facility and prevent contamination of the diets and of the rearing environment, specific biosecurity protocols are followed at all times (16). Sterilisation of pupae or adults also follows specific irradiation protocols aimed at inducing the required sterility without compromising the quality of the irradiated insects. Before irradiation, insects are marked with a specific fluorescent dye that allows to differentiate released sterile insects from wild insects when they are caught in traps (17). Biosecurity protocols are in place that allow trace back of each batch of irradiated insects in case of the need to check for a faulty procedure (4). The possibility of any introduced pathogen, parasite, or other form of contaminant in the production process being undetected is, therefore, extremely low.

The SIT programmes rely on the ability to measure and control the irradiation dose delivered to the insects for their sterilisation, to balance effective sterilisation with the utility of the intervention in terms of quality and vitality of the treated insects. Programmes have well established dosimetry systems to accurately measure the absorbed dose and estimate if the associated confidence interval is within the permissible range. This applies to any insect pest of agricultural, veterinary, and medical importance subject to sterilisation through ionising radiation. Procedures are available, such as the dose mapping by scanning Gafchromic® film, to measure the absorbed dose of insects during their sterilisation (18). This practically eliminates risks for end uses in terms of accidental introductions of insects that can establish and persist over generations.

Specific and rigorous quality control tests are applied to the mass rearing and sterilisation processes and to the insect itself aimed at

assuring high quality of sterile released insects. Standardised quality control procedures manuals are available for sterile insects used in operational programmes, such as the one for Tephritid fruit flies (19). In this way, the risk to the utility of the live insects is managed.

Post-production process

As in the case of the production process, the post-production handling of sterile insects is a delicate and rigorous process. Standard operating procedures are available to assure proper handling and good sterile insect quality. Strict biosecurity protocols are also in place in the emergence and release facility as well as for long distance transportation to assure the integrity of the consignment and prevent the accidental spread of unwanted insects (hitchhikers) (16, 22).

[Place Figure 2. here]

The need for international shipments for operational programmes

Production and sterilisation of sterile insects in centralised mass rearing facilities is a common practice (20). A centralised production and supply of sterile insects provides advantages such as more effective mass rearing and sterilisation processes, a more uniform quality of the sterile insects and a lower cost per million insects due to economies of scale (21). Nevertheless, in certain situations smaller local facilities ('The right size facility') may offer more flexibility, certainty in assuring the local availability of sterile insects, and resilience against factors such as problems with shipping. Sterile insects from centralised facilities can be used for field releases on site and/or to supply SIT programmes abroad through transboundary sometimes long-distance shipments. The largest insect mass rearing facilities in the world include the El Pino facility in Guatemala, with a production capacity of 2.5 billion sterile insects per week; the newly inaugurated facility in Metapa, Mexico, with a production capacity of 1 billion sterile insects per week; and the facility in Valencia, Spain, with a production capacity of 700 million sterile insects per week. All three facilities produce sterile Mediterranean fruit fly. These and other facilities have been a

source of sterile fruit flies for many years, shipping live insects as eggs, pupae, and adults across international borders to be used in SIT programmes in other countries (20).

Since the mid-1950s, when the use of SIT in operational programmes started, more than one trillion sterile insects have been shipped across borders to supply programmes in 31 recipient countries from 16 sterile insect production facilities based in 11 countries (DIR-SIT at <https://nucleus.iaea.org/sites/naipc/dirsit/PublicPages/Home.aspx>). On the list at this database are shipments of large volumes of several insect species, such as the New World Screwworm (*Cochliomyia hominivorax*), Medfly (*Ceratitis capitata*), Mexican fruit fly (*Anastrepha ludens*), tsetse fly (*Glossina spp*), pink bollworm (*Pectiniphora gossypiella*), and smaller shipments of other insect species such as codling moth, cactus moth and mosquitoes, listed in Table 1. This has been mostly done under the framework of cooperative agreements between countries and under technical cooperation projects of the United Nations.

[Place Table I. here]

Nevertheless, in recent years with the increasing interest in the use of SIT against major insect pests and the involvement of public-private insect mass rearing and sterilisation facilities, transboundary shipments of sterile insects outside the government programme framework have been conducted mainly for pilot trials. These shipments have faced significant constraints with the plant regulatory authorities in some countries as well as with the transportation companies which are confronted with a new and uncertain situation. This has significantly delayed some shipments and even prevented some shipments.

Harmonised guidelines adopted by international conventions such as the International Plant Protection Convention (IPPC) and World Organisation for Animal Health (OIE) would serve as a framework to both government and non-government organisations and agencies and would very much facilitate transboundary shipments of sterile insects, and, with it, foster the expansion of the SIT to more countries and for more insect pests.

Regulations

The SIT is a technology that has been recognised by the IPPC as a phytosanitary measure for pest control. SIT is defined as a ‘Method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species’ (22) and sterile insects are defined as ‘An insect that, as a result of a specific treatment, is unable to reproduce’ (22). Moreover, in its scope ISPM 3 (22) makes a distinction between biological control agents capable of self-replication (including parasitoids, predators, parasites, nematodes, phytophagous organisms, and pathogens such as fungi, bacteria, and viruses), and insects and other beneficial organisms that are sterile. The fact that sterile insects are not capable of self-replicating represents a major advantage to protecting the environment compared with other insects that are shipped across borders for biological control or other purposes.

A hazard analysis was conducted in 2001 by an FAO/IAEA Consultants Group to determine the level of risk of potential hazards from the transboundary shipments of sterile insects, including: 1) Outbreak of target insect pest in a new area, 2) Increase of fitness of local pest population, 3) Unnecessary regulatory action initiated, and 4) Introduction of exotic (new) contaminant organisms. The study concluded that the present systems of transboundary shipment of sterile insects for SIT programmes are very safe. Nevertheless, the study recommended that a harmonised guideline in the form of an international standard should be developed for approval by the IPPC Commission on Phytosanitary Measures (CPM) to facilitate commercial development of the SIT (4).

For insects that may be plant pests, the National Plant Protection Organization (NPPO) of each country should designate the proper authority for assuring safe shipment of sterile insects (either through or to their territory). It is up to the NPPO to coordinate with the producer/shipper regarding their responsibilities for achieving safe shipment, because producers of sterile insects may have diverse status,

including government as well as private, parastatal, joint venture, or internationally owned facilities.

Historically, the transboundary shipment of sterile insects has been subjected to simple basic phytosanitary regulations and documentation. This includes an import permit, national transit permit, phytosanitary certificate, irradiation certificate, labelling and notification, and transit regulations should the shipment transit through a third country (i.e., a country that is neither the country of origin nor the country of destination of the consignment) (Figure 3). The International Standard on Phytosanitary Measures (ISPM) No. 3 'Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms' states in the Section on 'Responsibility of Importers' that importers should ensure that organisms for SIT have been treated to achieve the required sterility for SIT purposes (e.g. using irradiation with the required minimum absorbed dose). The treatment(s) used and an indication of the effectiveness of sterilisation should also be provided (22).

[Place Figure 3. here]

The document titled *Guideline for Packing, Shipping, Holding and Release of Sterile Flies in Area-Wide Fruit Fly Control Programmes* presents a chapter describing the regulatory procedures involved in transboundary shipments of sterile insects including the responsibilities of the producer/shipper of sterile insects, of the authorities prior to export and upon import (final or transit) and the responsibility of the importer (6). No shipment of sterile insects has ever been rejected by national or international plant protection or regulatory authorities (23).

Future perspectives

Future investment in SIT may be focused on large centralised sterile insect production facilities from where insects can be shipped abroad to supply the needs of countries interested in applying the technology. This would avoid, when appropriate, the need for each country building smaller facilities to satisfy their needs (20, 24). Advantages of such a scheme are twofold: it would reduce the risk of non-native insects

escaping from in-country breeding colonies prior to sterilisation; and would allow greater control of the quality of the sterile insects, a critical aspect for effective application of SIT. Sterile insects would be available at more competitive prices, as insect mass rearing is susceptible to economies of scale (21). Nevertheless, an alternative scheme where smaller 'right size' facilities are built in the user countries, based on local needs, could provide greater flexibility and certainty in securing the required numbers of sterile insects, especially if there are uncertainties around the reliability of international shipment. This approach would require significant capital investment for each facility.

In order to take advantage of larger centralised facilities, an international framework is needed for transboundary shipments and importation (as a consignment in transit or for entry to the country of destination) of sterile insects for use in control programmes. Such a framework should recognise the extensive record of quality control and safe shipments of mass reared insects sterilised through ionizing radiation. This would serve as the basis for more harmonised regional or national phytosanitary and zoosanitary measures (23) and should apply for both government and private entities which follow the same procedures.

Conclusions

The SIT is a cost-effective and environment friendly pest control tool that has been extensively applied to control some major insect pests. Shipments of sterile insects across borders have been done for more than 65 years, requiring only the basic phytosanitary regulations.

Risks from the production phase are managed through quality control and agreed SOPs covering key steps. The fact that these insects are sterile essentially eliminates concerns around introductions of invasive or exotic species. No major incidents associated with transboundary shipments of sterile insects have occurred since the activity began in the 1950s.

Considering the negligible risk that has been demonstrated through many years of use, phytosanitary regulations for transboundary shipments of sterile insects should be kept as simple as possible. A harmonised framework to facilitate shipments of sterile insects across borders, regardless of the parties involved (public or private) would facilitate the expansion of the use of this effective and environment friendly technology.

Acknowledgements

The authors would like to acknowledge the managers of sterile insect production facilities for facilitating the data on the numbers of sterile insects shipped across borders in support of pest control programmes in many countries.

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Table I**History of transboundary shipment of sterile insects (updated from FAO/IAEA/USDA 2019)**

Year	Species	Site of production	Approximate amount shipped (million pupae)	Recipient	Purpose
1963–2000	Mexican fruit fly, <i>Anastrepha ludens</i>	Mexico (Monterrey and Metapa)	2,511	USA (Texas and San Diego)	To assist the California and Texas Departments of Agriculture in the eradication of Mexican fruit fly outbreaks
1970–2021	Mediterranean fruit fly, <i>Ceratitis capitata</i>	Argentina (Mendoza), Austria (Seibersdorf), Costa Rica, Guatemala (El Pino), Israel (Biofly), Mexico (Metapa), Portugal (Madeira), Spain (Valencia)	911,536	Argentina (Patagonia), Bolivia (Cochabamba), Chile, Croatia, Ecuador, Italy (Procida), Greece, Guatemala, Honduras, Israel, Jordan, Mexico, Morocco, Nicaragua, Spain (Canary Islands), Tunisia, US	For prevention, containment, suppression, and eradication of the Mediterranean fruit fly
1990–1991	New World Screwworm (NWS) <i>Cochliomyia hominivorax</i>	Austria (Seibersdorf), Mexico (Tuxtla Gutierrez, Chiapas), Panama (COPEG)	108,994	Central American countries (7 countries), Libya (Tripoli), USA (Florida)	To eradicate NWS from Central America To assist the eradication of NWS outbreaks in Libya and Florida, USA
1990–2021	Tsetse fly (<i>Glossina</i> spp.)	Austria (Seibersdorf), Slovakia	25.5	Tanzania (Tanga) Senegal (Dakar)	To assist Tsetse eradication from Tanzania and Senegal
2008	Cactus moth (<i>Cactoblastis cactorum</i>)	USA (Florida)	0.0213 ²	Mexico (Yucatan Peninsula)	Eradication of incipient cactus moth outbreaks

2001–2014	Pink bollworm (<i>Pectinophora gossypiella</i>)	USA (Arizona)	Unknown	Mexico (Northern States)	Regional (USA and Mexico) eradication of the pink bollworm
2010–2020	Codling moth (<i>Cydia pomonella</i>)	Canada (Osoyoos)	Unknown	New Zealand, South Africa, USA	For SIT pilot validation
Total			1,023,066.5		

Pre-print



Fig. 1
Metal box containing sterile fruit flies being loaded to an airplane for aerial release (photo Moscamed Programme Guatemala)



Fig. 2
Boxes containing sterile insects properly labelled for shipping (photo Moscamed Programme Guatemala)

CERTIFICADO DE ORIGEN
F No. 437172

LA CÁMARA DE COMERCIO DE GUATEMALA, hace constar:
a) Que el día de hoy se ha presentado a sus oficinas a requerir la emisión del presente certificado, el señor
[REDACTED] quien manifiesta estar en control del PROGRAMA MOSCAMED
comerciante domiciliado en: CALLE 25 SUR EN LA ZONA 10

PLANTA EL PINO
BARBERENA SANTA ROSA GUATEMALA, CENTRAL AMÉRICA, PAÍS (informe declaró que las necesidades abajo especificadas enviadas por vía
AEREA en el COPA CARGO con destino a [REDACTED])

MINISTERIO DE AGRICULTURA Y GANADERÍA, QUIMÚ, ECUADOR
a la orden de AGENCIA DE REGULACIÓN Y CONTROL FITO Y ZOOSANITARIO
con destino a [REDACTED]

CANTIDAD	DESCRIPCIÓN	VALOR UNITARIO	VALOR TOTAL
2	PAQUETES CENTENARIOS 3.500 MILLONES PUPA HACHO BETUL DE MOSCA DEL HEDERABAND.	ALZ0185	8000000
			\$25.00

PACTURA RC-52
VENTE Y CINCO DOLARES CON 00/100

19 AGO 2021

COMERCIAL DEPARTAMENT

PLANTA EL PINO
PROGRAMA MOSCAMED
GUATEMALA, C.A.
CERTIFICADO DE IRRADIACIÓN

CERTIFICADO DE IRRADIACIÓN
Cepa TSL

SE CERTIFICA QUE LAS BOLSAS DE MOSCA DEL HEDERABAND (Wiedemann)
FUERON IRRADIADAS DE ACUERDO CON EL PROCEDIMIENTO APROBADO

FECHA DE IRRADIACIÓN:	24/08/2021
TOTAL DE CAJAS:	2
BOLSAS IRRADIADAS:	18
BOLSA CENTRAL DE ESTERILIZACIÓN APLICADA:	145 Gy
NÚMERO DE ENVÍO:	EC 52 Ecuador
HORA DE HIPONIA:	8:00 24/08/21
HORA DE IRRADIACIÓN:	16:50 24/08/21

OBSERVACIONES:
LAS BOLSAS 01 A LA 10 VAN EN LA CAJA 01 Y LAS BOLSAS 11 A LA 18 VAN EN LA CAJA 02. ATENCIÓN: LA BOLSA 15 LLEVA 30000 PUPAS CON DESTINO A LA ISLA SANTA FLOREANA, Y LA BOLSA 16 LLEVA 15000 PUPAS CON DESTINO A LA ISLA ISABELA, LA BOLSA 17 LLEVA 30000 PUPAS PARA LA ISLA SANTA CRUZ Y LA BOLSA 18 LLEVA 30000 PUPAS PARA LA ISLA DE SAN CRISTÓBAL.

*LAS BOLSAS NÚMERO 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 FUERON TOMADAS COMO MUESTRA PARA CONTROL DE CALIDAD EN PLANTA EL PINO

CHEQUEO POR: [REDACTED] APROBADO POR: [REDACTED]

FIRMA: [REDACTED] FIRMA: [REDACTED]

radiación y empaque • PINO: (010 244 1210) • cepa@pino@netfy.org.gt

Fig. 3
Official documents used for transboundary shipment of sterile insects (certificate of origin and radiation certificate) (photo Moscamed Programme Guatemala)
 Signatures and names redacted for data protection purposes