APPENDICES

Appendix A: Chronology of Product Quality Control of Tephritid Flies for Use in SIT Programmes

1977 Publication of "An Idea Book for Fruit Fly Workers" (Boller and Chambers 1977), which includes many original papers and bibliographic references on how to measure overall performance, individual traits, and production of laboratory-reared tephritids.

1978 IOBC Palearctic Working Group on Fruit Flies of Economic Importance met at Sassari, Sardinia, Italy where quality control of tephritid fruit flies was proposed as a discipline.

1978 Boller, Calkins, Chambers, Cunningham, Greany, Hendrichs, Huettel, Leppla and Ruhm meet in Guatemala under the auspices of IAEA, USDA, ARS and Moscamed to conduct laboratory and field tests of quality control on laboratory and field collected medflies.

1979 Lek mating system described in medfly (Prokopy and Hendrichs 1979). International Organization of Biological Control (IOBC) sponsors a course in Castellon, Spain and a manual (Calkins et al. 1979) to cover methods for assessing pupal size, flight ability, startle activity, olfactometry, mating propensity, dispersal and survival, and ratio tests.

1981 Publication of the RAPID methods and apparatuses for "Measuring, Monitoring, and Improving the Quality of Mass-Reared Medflies" (Boller et al. 1981). This publication suggests that five tests, i.e., pupal size, flight ability, startle activity, response to pheromone, and mating propensity, be carried out at frequent intervals.

1981 An International Technical Group on Quality Control meets in Guatemala to standardize basic methods and tests. Six tests are recommended that should be carried out regularly and nine others as time and resources permit (Klee 1981). Procedures and samples of reporting forms to be used were included.

1982 First mating compatibility test on a field caged host tree, measuring female choice by allowing wild female flies to select among competing wild and sterile mates (Zapien et al. 1983).

1982 IOBC Global Working Group on Quality Control of Mass Reared Insects was formed. The first meeting was held in Gainesville, Florida, USA. Subsequent meetings were held in Wadenswil, Switzerland in 1984, in Guatemala City, Guatemala in 1986, in Vancouver, British, Columbia, Canada,in 1988, Wageningen, The Netherlands in 1991 in Rimini, Italy in 1994, and in Santa Barbara, California, USA in 1996.

1982 International Symposium on Fruit Flies of Economic Importance had a section on Quality Control, Athens, Greece. Also held in 1986 in Crete, 1990 in Antigua, Guatemala, and 1994 in Clearwater, Florida, USA.

1983 Field tests for extending and confirming the results of laboratory data from RAPID tests are published by Chambers et al. (1983). The staff at the Moscamed Programme in Mexico, publishes a manual of >40 lab and field tests that have been used extensively to measure the quality of mass produced flies in Latin America (Orozco et al. 1983).

1984 USDA, IAEA and Moscamed Guatemala establish a cooperative pilot test of a systematic process, based on the RAPID tests, for measuring and controlling quality through process control, product evaluation, and data management. A manual for laboratory QC of mass-reared Medflies is developed by C. Calkins and T. Ashley.

1986 USDA-APHIS compiles and distributes "Required quality control tests, quality specifications, and shipping procedures" (Brazzel et al. 1986). This manual was designed to ensure that Mediterranean fruit flies for SIT programmes with USDA involvement meet certain quality standards. The QC tests included were primarily a check on the rearing process and could all be carried out with inexpensive (or easily constructed) equipment in a minimum of laboratory space. This has been the QC guide followed in USDA, Moscamed, and CDFA rearing facilities for the past 10 years.

1989 A case of behavioural resistance (to mating with sterile flies) is documented for the melon fly in Okinawa Islands by Hibino and Iwahashi (1989, 1991). A doubtful case was later reported for the medfly from Hawaii (McInnis et al. 1996).

1994 The Insect Pest Control Section of the Joint FAO/IAEA Division for Food and Agriculture launches a Coordinated Research Project to study medfly courtship and female choice behaviors, to assess the compatibility of medfly populations from different origins world-wide, and to standardize field cage mating compatibility tests.

1996 Indices (*RII*, *RSI*) to measure the mating competitiveness in field cage test are published (McInnis et al. 1996).

1997-98 19 experts from several countries gathered in Vienna for one week to harmonize internationally fruit fly product quality control, and to agree on the manual of "Product Quality Control, Irradiation and Shipping Procedures for Mass-Reared Tephritid Fruit Flies for Sterile Insect Release Programmes". The FAO/IAEA/USDA manual of "Product Quality Control, Irradiation and Shipping Procedures for Mass-Reared Tephritid Fruit Flies for Sterile Insect Release Programmes" (Version 4) is released in September and recognised as an International Guideline for fruit fly SIT projects.

1999 New indices (*ISI*, *MRPI*, *FRPI*) to measure the mating competitiveness in field cage test are published (Cayol et al. 1999). These indices were included in Version 4.0 of the present manual. The Insect Pest Control Section of the Joint FAO/IAEA Division for Food and Agriculture launches a Coordinated Research Project on "Quality Assurance of Mass Produced and Released Fruit Flies" with the objective to further improve and standardise international quality control procedures.

2001 The revision of the FAO/IAEA/USDA manual of "Product Quality Control and Shipping Procedures for Sterile Mass-Reared Tephritid Fruit Flies" (Version 5) is initiated.

Note: the references quoted in this section can be found in sections 1.5 and 3.3 RELEVANT LITERATURE of this manual.

Appendix B: Known Sources of Key Equipment and Supplies[14]

Item	Source
As described in 2.1 PUPAL SIZE TEST	
Roller gauges [for pupal sizing and separating, (“*puppentransporteinrichtung*”)]	**Ing. Alfred PARAL** Postfach 27, Hainfelder Strasse, A-3071 Boeheimkirchen, Austria. Fax: (+43) 274323045
Seed Counter [for counting pupae, ELMOR 600/A05 with screw on conveyor bowl including discharge chute and foot actuator]	**Dr. Rudolf MOLL** Export Department Mangelegg 58, CH-6430 Schwyz, Switzerland. Fax: (+41) 43216508
As described in 2.2 PERCENT EMERGENCE AND FLIGHT ABILITY	
Tubing for Flight Ability tubes [Tube-Pak, size No. 52D I.D. 3 ¼", O.D. 3 ½", 8 ft length, Part No. 06048C]	**Consolidated Plastics Company** 9085 Freeway Drive, Macedonia, Ohio 44056, USA.
As described in 3.1 MATING PERFORMANCE FIELD CAGE TEST	
Day-Glo powder [example: Blaze Orange, reference JST43]	**Radiant Color** Europarklaan 80, B-3530 Houthalen, Belgium Tel.: (+32) 11520760, Fax: (+32) 11526679 E-mail: info@radiantcolor.be
Field Cages [20 x 20 HDPE Screen fabric - 2.9 meters diameter x 2.0 meters height with floor and one 2-way zipper (bottom to top)]	**Synthetic Industries** P.O. Box 977, 2100A Atlanta Highway, Gainesville, Georgia, 30503, USA. Fax: (+1) 7705311347
Lightmeter and datalogger [model Testo 545 (lightmeter) and Testostor 171 (datalogger)]	**Testo GmbH & Co.** Postfach 11 40, Testo-Strasse 1, D-79853 Lenzkirch, Germany Tel.: (+49) 7653681-0, Fax: (+49) 7653681-100 E-mail: info@testo.de, Website: www.testo.de
As described in 3.2 RELEASE-RECAPTURE TESTS OF DISPERSAL AND SURVIVAL	
Attractant [BioLure® Three-Component Fruit Fly Lure (Triple Pack) (trimethylamine, putracine, and ammonium acetate)]	**Suterra** (formerly Consep, Inc.) 213 S.W. Columbia St., Bend OR 97702-1013, USA. Tel.: (+1) 5413172254, Fax: (+1) 5413883705

[14] *Reference to any commercial product or service is made with the understanding that no discrimination is intended and no endorsement by the FAO/IAEA or the United States Department of Agriculture is implied. Reviewers are strongly encouraged to contribute with additional sources of equipment and supplies.*

Item	Source
Attractant [cue-lure]	**Scentry Biologicals Inc.** 610 Central Venue, Billings, MT 59102, USA. Tel.: (+1) 4062485856, Fax: (+1) 4062452790
Attractant [methyl-eugenol (eugenol methyl-ether)]	**Agrisense - BCS Ltd.** Treforest Industrial Estate, Pontypridd Mid Glamorgan CF37 5SU, United Kingdom. Tel.: (+44) 1443841155, Fax: (+44) 1443844205 **American Scientific & Industrial Supplies** PO Box 8247, Radnor PA 19087, USA. Tel.: (+1) 6109647665, Fax: (+1) 6109641860 E-mail: sales@asi-supplies.com
Attractant [torula yeast pellets]	**Scentry Biologicals Inc.** *(see above)*
Attractant [trimedlure]	**Better World Manufacturing Inc.** 5690 E. Dayton Avenue, Fresno CA 93727, USA. Tel.: (+1) 3055958911, Fax: (+1) 3055957806 E-mail: multilure@aol.com
Ultra-Violet Lamp [High Intensity Long-wave. B-100AP]	**Ultra-Violet Products (UVP) Inc.** 2066 W. 11th Street, Upland, CA USA. Fax: (+1) 8004526788 or (+1) 9099463197 E-Mail: uvp@uvp.com European Sales Operations: **Ultra-Violet Products Ltd.** Science Park, Milton Road, Cambridge, CB4 4FH, United Kingdom. Tel.: (+44) 1223420022 E-mail: uvpuk@uvp.com
Ultra-Violet Lamp with magnifier [Philalux II HF, Art. 9865]	**Schwaebische Albumfabrik GmbH & Co.** P.O. Box 60, D-7445 Bempflingen, Germany. Fax: (+49) 712332550
Traps and trapping supplies	**Better World Manufacturing Inc.** *(see above)* **Gempler's Pest Management Supply** P.O. Box 270, 211 Blue Mounds Rd., Mt. Horeb, WI 53572, USA. Tel.: (+1) 8002727672, Fax: (+1) 8005511128 **Scentry Biologicals Inc.** *(see above)* **Sorygar S.L.** (for Tephri® traps) Quinta del Sol n. 37, Las Rozas - Madrid 28230, Spain. Fax: (+34) 916407000 E-mail: sorygar@nexo.es **Suterra** (formerly Consep, Inc.) *(see above)*
As described in 4.3 IRRADIATION AND PROCESS CONTROL	
Dose-specific radiation-sensitive indicators [70, 125, 300 Gy]	**International Speciality Products (ISP) Technologies Inc.** 1361 Alps Rd, Wayne, New Jersey, 07470, USA. Tel.: (+1) 2016284000, Fax: (+1) 2016283016

Item	Source
As described in 5.1 Dosimetry System	
Gafchromic® dosimetry media [type HD-810, package of 5 sheets, 8" x 10 "]	**Elimpex Medizintechnik GesmbH** Spechtgasse 32, A-2340 Moedling, Austria. Tel.: (+43) 2236410450, Fax: (+43) 2236410459 E-mail: falk@elimpex.com (specify "catalog number 37-040") **ISP Technologies Inc.** *(see above)*
Digital Radiachromic® Reader System [model FWT-92D-220]	**Far West Technology Inc.** 330 D South Kellogg, Goleta CA 93117, USA. Tel.: (+1) 8059643615, Fax: (+1) 8059643162
Miscellaneous	
Fluorescent microscope [trinocular with phase contrast, model VanGuard 1486FL]	**Service for Science and Industry (SFSI) Inc.** 1101 North Kings Highway, Suite 201, Cherry Hill NJ 08034, USA. Tel.: (+1) 8563210635, Fax: (+1) 8563210636 E-mail: sfsi@sfsi-usa.com
Multi-use temperature and/or humidity monitors	**Temperature Data Systems** Wattstraat 68, P.O. Box 168, 2170 Ad Sassenheim, The Netherlands. Tel.: (+31) 0 252211108, Fax: (+31) 0 252231032 Website: www.temperaturedatasystems.com

Appendix C: Ancillary Tests

Laboratory Mating Test

Objective

The laboratory mating test provides information of the basic ability of flies to mate, albeit under controlled, artificial conditions.

Discussion

Inadvertent selection for insects that don't attempt to mate would seem unlikely in a large laboratory colony. Because of that, performance of flies in this test should be viewed as an indicator of overall fly quality as affected by the rearing process. The test will not detect many factors that may reduce the acceptability of sterile males to wild females. Thus, high scores on this test carried out under high densities in small cages cannot be considered evidence that sterile males will perform well in the field. In contrast, for a given strain of flies from a given production facility, downward trends in scores on this test is a reasonably certain sign that flies will not perform well in the field.

The laboratory mating test can be used as a routine indicator that the sterile flies going out into the field are at least capable of mating.

Equipment

- Plexiglas mating cages: 30 x 40 x 30 cm (w x d x h) with a access hole, covered with fine nylon mesh or surgical stocking and a ventilation hole covered with 16 mesh screen.
- Aspirator.
- Environmentally controlled space.

Test conditions

- temperature: 25 ± 1° C
- humidity: 65 ± 15% RH
- light: 2000 lux (*C. capitata*)
  200 lux (*A. ludens*)
  2500 lux (*A. suspensa*)
- photo-period 14:10 Light:Dark (for holding flies for sexual maturation)

Note: For standardization, the light source should ideally be a daylight-balanced tungsten light with a bare bulb (e.g., Phillips incandescent daylight blue, 17.5 lumens/watt) above each cage. With some lighting set-ups, interiors of cages may warm during the course of the tests. This should be minimized by not placing cages on warm surfaces and, if necessary, by rotating the cage 90° so that the screened opening is on the side rather than the top. Tests may have to be started at slightly cooler than the desired temperature in order to achieve an average of 25° C during the test.

Procedure

Flies should be separated by sex within a few hours of adult emergence and provided with food (protein:sucrose 1:3) and water until sexually mature (use of flies of a standardized age is recommended). Time of day when the test is run will vary with species. For *C. capitata*, the test is begun as soon as possible following a period of at least 10 hours of darkness. For *A. ludens*, the test is begun after 12 hours of photophase (daily light:dark cycles under which the flies are held before the test may be adjusted to, say, "lights on" at 0300 hours and "lights off" at 1700 hours to allowing tests to be run during normal working hours). For *A. suspensa*, tests should be started 10 hours after "lights on". Transfer 25 males and then 25 females within 5 minutes into each of 5 mating cages. Allow flies to mate for 1 hour, periodically removing and counting mating pairs. Compute percent of flies mating for each cage.

Note: In its original form, this was known as the mating propensity test and produced an index that was sensitive to both the incidence and speed of mating. Mating pairs were counted and then removed from the cage at the end of each of 6 consecutive 10-min intervals (a total of 1 hour). Numbers of couples mating in the 1^{st}, 2^{nd} to 6^{th} intervals were then multiplied by 100, 50, 33, 25, 20, and 15, respectively. The sum of these products was then divided by the total number of pairs in the cage (25, barring accidental death, escape, etc.) to yield the mating propensity index. It was later shown that the mating speed is lower in wild flies and therefore often only reflects the adaptation of mass reared males and females to laboratory conditions but, by no means, could reflect the behavioural quality expected from mass reared males when courting wild females. In addition, flies which do perform mating well and fast under the restrictive and artificial conditions of the laboratory mating test, usually exhibits relatively low mating competitiveness when competing with wild males for wild females in outdoor or open field cage conditions.

Interpretation

For a given strain and rearing system, downward trends in the percentage of flies mating over time may be indicator of problems in rearing or handling of flies. For thoroughly lab-adapted strains, well over 50% of healthy flies, on the average, will typically mate within the test period. Typically, percentages of flies mating will be relatively lower for *P*-generation wild flies or flies from recently colonized laboratory strains.

The Fried Test

Objective

To provide an estimate of the overall mating competitiveness of sterile male fruit flies.

Discussion

An index of mating competitiveness, *C*, is based on the degree of sterility in eggs produced by wild females when sterile males and wild males compete for these wild females. The mating competitiveness of sterile males will influence the number of sterile males required for releases to induce the target degree of sterility into the wild population. In mass rearing, there are many factors that can change the mating competitiveness of an insect. Fried (1971) considered that final expression of mating competitiveness could be measured in the egg sterility induced in the population. He presented a model for determining mating competitiveness using different ratios of sterile to wild males. This was adapted into a test for mating competitiveness and has subsequently been called the Fried Test. The Fried test has been conducted in lab/field cages (and open field experiments) for many years in some facilities.

Sources and Handling of Flies

a) ***Sterile laboratory males:***

As for compatibility test, but do not mark (paint) flies.

b) ***Fertile laboratory females:***

Place several thousand *non*-irradiated, unmarked pupae in a cup in a screen or Plexiglas cage and separate sexes within a few hours of emergence. Then, hold the flies until sexually mature in laboratory cages (screen or Plexiglas) containing an appropriate source of food and moisture (e.g., water and 1:4 mixture of protein:sucrose). Age of sexual maturity will vary with species and strain.

c) ***Wild flies:***

As for compatibility test, but do not mark (paint) flies.

Equipment

- Outdoor field cage containing a host tree, as for the compatibility test.
- Oviposition substrate, agar egging balls or ripe host fruit (free of tephritid eggs).
- Dissecting microscope.
- Dissecting forceps.
- Two laboratory oviposition cages; one for testing control egg hatch from wild females, and one for testing the sterility of irradiated males.

Materials and Methods

a) ***Competitiveness field cage test:***

Before the field cage test (at least the proceeding day), flies are transferred to containers suitable for releasing them into field cages in groups of 20 or 40 flies per container (flies are provided with food, moisture, and ventilation in the containers). Add 16 oviposition substrates to the field cage, by hanging them in the fruit tree on eight equidistant compass points at 2 different levels in the tree. On the day of the test, 40 wild males and 120 sterile males with 40 wild females are released into the field cage. Food and water are added to the field cage for the duration of the test.

b) ***Sterility test cage:***

25 sexually mature sterile males and 25 sexually mature fertile laboratory females are placed into an oviposition cage with food and water. The oviposition substrates are added to the cage at the same time as for the field cage.

c) ***Control test cage:***

25 wild sexually mature males and 25 wild sexually mature females are placed into the other oviposition cage with food and water. This cage can be placed in the laboratory if necessary. The oviposition substrates are added to the cage at the same time as for the field cage.

d) ***Procedure:***

The test is run for 4 days. At 48 hours after placing the flies into the respective field cages, infested agar balls are replaced with a new set of balls. The infested balls are dissected within 2 hours of removal. Eggs from the ball are placed onto moistened black filter paper in a 9-cm Petri dish. The eggs are incubated at 25°C and after 5 days, the percentage egg hatch is evaluated. For meaningful data, at least three replicates of this test are required.

Interpretation

The Fried competitiveness value (*C*) is computed using the following formula:

$$C = \frac{W}{S} x \frac{Hw - Hc}{Hc - Hs}$$

In this formula, *W* is the number of wild males in the competitiveness cage, *S* is the number of sterile males in the competitiveness cage, H_w is the egg hatch from wild females in the control cage, H_c is egg hatch from wild females in the competitiveness cage and H_s is the egg hatch from lab females in the sterility cage.

Normally, *C* varies between 1 and 0. Values of 1 indicate equal competitiveness between sterile and wild males. It is not uncommon to record values of more than 1; therefore, replicates with values greater than 1.1 should be discarded, and values between 1 and 1.1 should be rounded down to 1.0. Values between 0.2 and 0.4 are normal for sterile lab males.

Pheromone Compatibility Test

Objective

To determine if the pheromone produced by males that were mass reared for sterilization and release is attractive to wild females.

Discussion

Frequently, colonies of fruit flies are cultured in the rearing facilities for many generations without any influx of new genes. Mating occurs under extremely crowded conditions and there is some question whether pheromone is used in the courtship process. There is the possibility that pheromone production may be reduced under this circumstance. There is also the possibility that the ratio of the components of the pheromone may vary or change. If any of these situations occur, this could affect how the wild target female would react to the sterile male in the mating arena. The following describes a simple test that does not have to be conducted more frequently than once each year during the time when the mating compatibility tests are being conducted.

Source and Handling of Flies

a) ***Sterile flies:***

Place several hundred irradiated pupae in a cup in a screened or Plexiglas cage and separate the sexes within a few hours of emergence. Flies are held in a screened cage containing water and a protein:sucrose (1:3) diet until they are sexually mature. See also 3.1 MATING PERFORMANCE FIELD CAGE TEST.

b) ***Wild flies:***

Wild flies are collected as larvae in fruit. The fruit is held in the laboratory for larval emergence and are placed in a pupation media. When flies emerge, they are sexed and handled as the sterile flies above. See also 3.1 MATING PERFORMANCE FIELD CAGE TEST.

Equipment

- Standard round outdoor field cages with host trees.
- Nine small-screened cages (10x10x10 cm) with 2/3 m long cord and hooks on ends to suspend cages inside the large field cages.
- Aspirator.
- Clipboard and forms.

Procedure

Place 5 sterile males in each of 3 small cages, 5 wild males in each of 3 small cages and keep 3 small cages empty. Suspend small cages from branches in the shade of the host tree(s) equal distance from each other, about 1/3 m from the side of the cage and about 2/3 m from the top. Cages containing different treatments should be alternated so that no two cages containing the same treatment are adjacent to each other. Release 100 wild females inside the field cage. After 10 minutes, count the number of female flies on screens of each cage and record the number on the form. Then gently blow the female flies from the small cages and rotate the cages one stop. Repeat the test every 10 minutes for 9 periods so that each small cage has occupied each position in the large cage. Total the number of females for each treatment and determine the mean and the standard error.

Interpretation

Based on experience, one would expect the sterile males to call more than the wild males. If they produce similar amounts of pheromone as the wild males, one would therefore expect more wild females on cages containing sterile males. The empty cages should have no or very few females resting on them. If there are substantially more females resting on the wild male cages than on the sterile male cages, there may be some concern that the pheromone has either changed or the sterile males are not producing enough pheromone. This could conceivably affect the compatibility of the sterile male and the wild female in the field.

Appendix D: Terminology

Accessory glands - glandular structures associated with the spermatheca that produces a material that accompanies the sperm during ejaculation.

Adjacent-1 - genetic sexing strains that have a Y-linkage, produce adjacent 1 segregation at meiosis. This produces 2 classes of genetically unbalanced gametes. One class is characterised by a deletion of one part of the Y-linked autosome, while the second class contains the other part of the respective autosome in triplicate. In the latter, a few individuals survive to adulthood. Characteristically, adjacent 1 individuals exhibit lower emergence. Furthermore, there is no evidence that the adjacent-1 individuals reproduce (i.e. in most cases they are too weak to mate and their longevity is reduced dramatically.)

Aedeagus - the male sexual organ used to transfer sperm to the female.

Assortative mating – term used to describe a tendency for male/female of a given population to preferably mate with male/female of the same population.

Calling - the act of dispensing pheromone by the male fruit fly to attract the female.

Compatibility (mating) - term used when females of a given strain are able and willing to accept, for mating, the males of another strain; this also includes synchrony and other factors that cause reproductive disconformancy.

Competition - interaction between organisms that share a limited environmental resource.

Competitiveness – ability of an organism to compete with conspecific organisms for a limited environmental resource.

Consignment –a quantity of plants, plant products and/or other biological articles being moved from one country to another and covered, when required, by a single phytosanitary certificate (a consignment may be composed of one or more commodities or lots).

Consignment in transit – a consignment which is not imported into a country but passes through it to another country, subject to official procedures which ensure that it remains enclosed, and is not split up, not combined with other consignments nor has its packaging changed.

Contaminating pest – a pest that is carried by a commodity and, in the case of plants and plant products, does not infest those plants or plant products.

Control chart - to plot a parameter with predetermined limits on a time scale and to present this information in an easy to interpret graphical form such as on mean- or range-charts that have control limit lines.

Copulation - a sexual union.

Courtship - the courting behaviour of male animals with the expectation of mating.

Data logger – a device used to record temperatures (or any other environmental variable) during a variable length of time.

Deformed (wings) - wings of flies that are not fully expanded or are bent or crumpled.

Diel periodicity – time of day at which an organism tends to exhibit a behaviour or trait.

Emergence (adult emergence) – the escape of the adult insect from the cuticle of the pupa.

End user - the agency or personnel that actually uses or releases the flies received from the producer.

Flight ability - adult capability to achieve a defined flight performance.

Irradiation certificate – document presented by the shipper to the importer to certify that the insects contained in the package were irradiated at a specified dose.

Lek - a communal display site where males aggregate for the sole purpose of attracting and courting females and to which females come for mating.

Lux - a unit of illumination equal to one lumen per square meter. The lumen is ca 1/683 Watt.

Mating pair - male and female flies in copula. This does not obviously include sperm transfer.

Mating system - the process of assuring that males and females of a given species interact sexually for reproduction. In the case of pest tephritid fruit flies, it is a female choice system.

Normal non-flier - a fly that appears to be normal but does not fly.

Over flooding ratio - the ratio of sterile flies to wild flies in the population in an SIT programme.

Process control – regulation of the performance of production processes through feedback so that deviations from product tolerances and specifications do not occur. Parameters within the realm of process quality control for fruit fly production include, among others, percent egg hatch, eggs per unit of diet, quantity of diet inoculated, percent pupation, age of pupae irradiated.

Production control – development, installation, and maintenance of methods used to produce a product at the greatest rate, most efficiently.

Product control – the composite product characteristics of production and testing to which the product in use will meet the expectations of the customer. Parameters within the realm of product quality control for fruit fly production include, among others, pupal weight, sex ratio, longevity, flight ability, pheromone production and response, mating propensity, and mating compatibility.

Pheromone - a chemical produced by one organism that influences the behaviour of another organism of the same species.

Partially emerged - a fly that has not completely emerged from the pupal case, ranging from only the head free to the case adhering to the abdomen.

Packing - the act of placing the pupae into a package and the package placed in a shipping container prior to shipping.

Propensity - an inclination or tendency; the tendency for an individual insect to carry out an act, or for an individual event to occur.

Quality - the degree to which a product meets the requirements of the objective or of the expected function.

Quality control – a systematic process where by management(s) critically evaluates the elements of production, establishes standards and tolerances, obtains, analyses, and interprets data on production and product performance, and provides feedback so as to predict and regulate product quality and quantity.

Range - the area between limits of variation especially as representing a scope of effective operation.

Recombination - the genetic exchange between two homologous chromosomes leading to the occurrence of recombinants. In case of genetic sexing strains, it refers primarily to recombination in males resulting, in the next generation, in a reversal of the sexing system, i.e. recombinants are either wild-type females or mutant males.

Remating - the act of a male or female mating again at some time after previous mating.

Refractory period - the period between matings, usually induced by a substance from the male that inhibits the female from mating.

SOPs - refers to various Standard Operating Procedures (however, each facility has also its SOPs for process qc, etc.) developed by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. The SOPs may be entitled as such or appear within other publications such as Quality Control Manuals, but must be recognized by this international organization.

Specifications - possessing or concerned with properties that characterize a factor used to describe a quality aspect.

Sperm transfer - the successful transfer of sperm from a male to a female spermathecae during copula. This may be accompanied by accessory gland fluid.

Standard - a quality or measure serving as a basis or principle by which others conform or should conform or by the accuracy or quality of others is judged.

Sterility – a condition where sperm or eggs from irradiated reproducing individuals do not result in viable progeny.

Strain - a breed or stock of fruit flies that have been held in isolated colonies for a period of time.

Target population - the wild population that the sterile flies are being released against.

Wild fly – a fly that has never been domesticated or held in a rearing colony.

Appendix E: History of Transboundary Shipments of Sterile Tephritid Fruit Flies

(1963-2000)

Year	Tephritid species	Site of production	Amount shipped *(million pupae)*	Recipient	Observations
1963-1990	Mexican fruit fly, *Anastrepha ludens*	Monterrey, Mexico	Unknown	Texas, USA	
1970/71	Mediterranean fruit fly, *Ceratitis capitata*	Seibersdorf, Austria	Unknown	Procida, Italy, and Greece	Relatively small amount since sterile flies were used for field trials
1970	Mediterranean fruit fly	Costa Rica	Unknown	Nicaragua	Relatively small amount since sterile flies were used for field trials
1975-1977	Mediterranean fruit fly	Madrid, Spain	302	Canary Islands	
1978	Mediterranean fruit fly	Seibersdorf, Austria	Unknown	Guatemala	Sterile pupae shipped from the IAEA laboratories (Seibersdorf) to a packing and emergence facility in Guatemala for field trials and staff training in SIT techniques
1979-2000	Mediterranean fruit fly	Chiapas, Mexico	280,000	Guatemala	Biweekly transboundary shipments have been carried out for the past 21 years
1989-1994	Mediterranean fruit fly	Chiapas, Mexico	6,670	California, USA	To assist the CDFA in eradication of medfly outbreaks
1990	Mediterranean fruit fly	Chiapas, Mexico	552	Chile	Sterile flies donated by the Mexican government to Chile
1989-1990	Mediterranean fruit fly	Seibersdorf, Austria	Unknown	Israel	Pilot trials
1994	Mediterranean fruit fly	Seibersdorf, Austria	60	Tunisia	Pilot trials
1996-2000	Mexican fruit fly	Chiapas, Mexico	2,511	California, USA	To assist the CDFA in eradication of Mexican fruit fly outbreaks
1994-2001	Mediterranean fruit fly	El Pino, Guatemala	51,800	California, USA	To assist the CDFA in eradication of medfly outbreaks
1997/98	Mediterranean fruit fly	Madeira, Portugal	206	Israel	In support of pilot suppression programme

Year	Tephritid species	Site of production	Amount shipped *(million pupae)*	Recipient	Observations
1997-2000	Mediterranean fruit fly	El Pino, Guatemala	1,000	Israel	In support of pilot suppression programme
1998-2001	Mediterranean fruit fly	El Pino, Guatemala	19,500	Florida, USA	To assist the State of Florida in eradication of medfly outbreaks
1999-2000	Mediterranean fruit fly	El Pino, Guatemala	600	South Africa	In support of pilot suppression programme
TOTAL			**363,201**		

Appendix F: Transboundary Shipments of Sterile Insects

Prepared by an FAO/IAEA Consultants Group
30 July to 3 August 2001, Vienna, Austria

PREAMBLE

A Consultants Group Meeting was held to discuss the potential risk[15] from transboundary[16] shipment of sterile insects for pest control programmes. This meeting took place in Vienna at the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, from 30 July through 3 August 2001. The group of consultants (see Annex 1) was called together in response to requests for guidance from national plant protection organizations (NPPOs) in light of the growing demand for alternatives to pesticide use as an exclusive control measure and the increasing interest from the private sector to invest in the Sterile Insect Technique (SIT).

The aim of the meeting was to characterize the potential risk posed by transboundary shipment of sterile insects shipped for SIT programmes and to reach conclusions regarding the level of risk. In the process of this analysis, the group identified some routinely applied procedures, including best practices for shipment that reduce the risk to a negligible level. However, there currently are no internationally recognized guidelines for regulating shipment of sterile insects.

Harmonized guidance regarding regulation of the shipment of sterile insects will facilitate trade while addressing concerns about shipment of what could be quarantine pests. This document was developed as a discussion paper for consideration by the Interim Commission on Phytosanitary Measures (ICPM), the governing body for the International Plant Protection Convention (IPPC).

One possible result of this discussion paper will be the development of an international standard providing guidance on measures pertaining to the transboundary shipment of sterile insects. Alternatively, this topic could be added to the International Standard on Phytosanitary Measures (ISPM) regarding biological control agents (IPPC, 1996) at the time of its revision. However, certain provisions in the ISPM on biological control agents are inappropriate when considering sterile insects (e.g. holding in quarantine for the next generation). In addition, the IPPC Glossary of Terms (IPPC, 2001) definition of biological control excludes the SIT.

In the interest of harmonization, similar discussions may be needed at the Office International des Epizooties (OIE) and the World Health Organization (WHO) regarding the use of sterile insects for control of human or animal diseases.

EXECUTIVE SUMMARY

- The increased use of the Sterile Insect Technique (SIT) to suppress or eradicate insect pest populations is resulting in increased shipment of the sterile target insect pests from one country to another, often passing in transit through other countries. These transboundary shipments are not subjected to international standards for biological safety.
- As the SIT becomes more commercial, the need for guarantees that the sterile insects can be safely and legally shipped are essential to encourage financial investments in commercial sterile insect mass rearing facilities. Also, international regulations are required to reduce the need for independent development of national regulations that may hinder the insect control programmes.
- The objective of the Consultants Meeting was to prepare a discussion paper for consideration of the Interim Commission on Phytosanitary Measures (ICPM), the governing body for the International Plant Protection Convention (IPPC), as a first step towards developing an international standard or other guidance on the transboundary shipment of sterile insects. Additional discussions may be needed to address shipments of sterile insects for control of pests of veterinary and medical importance.

[15] "Risk" in this context includes both the likelihood and the consequences of an adverse event occurring
[16] "Transboundary" in this context refers to entry (Customs and Agriculture clearance) of a shipment into the importing country as well as transit shipment through a third country. Transit may or may not involve transloading.

- The scope of the discussions was limited to radiation-sterilized insects for use in Sterile Insect Technique (SIT) control programmes against plant insect pests. Insect strains produced artificially by genetic engineering or other modern biotechnology methods were excluded.
- Four potential hazards were identified with regard to transboundary shipments of sterile insects:
  1. Outbreak of the target pest in a new area, where it does not already occur.
  2. Increase of fitness of the local pest population through the introduction of genetic material from the escaped insects into an area where the pest already exists.
  3. Unnecessary regulatory actions being initiated following false identification of captured sterile insects and conclusion that it is a quarantine threat.
  4. Introduction of exotic contaminant organisms in a shipment, other than the target species for the SIT programmes.
- Transboundary shipment of sterile insects has taken place on a continuous basis for nearly 50 years. The total number of sterile insects shipped was estimated at 962 billion in more than 12,000 shipments to 22 recipient countries from 50 sterile insect factories in 25 countries. During this long period and many precedents, no problems associated with the hazards listed above or any other have been identified, and thus the shipment of sterile insects have never been subjected to any regulatory action.
- The potential risks of the identified hazards were evaluated using a scenario analysis technique.
- The events considered for hazard 1, were: sterilization failure, shipment packages opened accidentally, escape, survival and reproduction of the sterile insects. For hazard 2, in addition to the above sequence of events, the escaped insects would have to reproduce with a local population and undesirable traits established in the population. For hazard 3, the critical points would be shipment packages opened accidentally, escape, survival and captured insects not recognized to be sterile. Hazard 4 is not unique to sterile insects and was thus not assigned a risk, as it is possible in shipments of goods of any type.
- For each hazard the calculated estimated risk was:
  1. $0.5x10^{-18}$
  2. $0.5x10^{-23}$
  3. $1x10^{-11}$
  4. Many-fold less likely than the risk of moving biological control agents
- It was concluded by the consultants that the present systems of transboundary shipment of sterile insects for SIT programmes is very safe. However, international regulations should be developed for approval by the Interim Commission on Phytosanitary Measures (ICPM) to facilitate commercial development of the SIT.

I. INTRODUCTION

There is a growing demand for cost effective control of insect pests of plants, as well as insects of veterinary and medical importance. At the same time insecticides are under greater scrutiny for potential toxicological and environmental impacts. An alternative insect pest control method is the Sterile Insect Technique (SIT). This involves mass production of the target insect species, sterilization using ionising radiation and repeated release into the target population. The release of sterile insects that target a population of the same species is a form of "birth control". The sterile insects mate with the wild population but fertilization results in no viable offspring. Repeated releases of sterile insects lead to a reduction in the pest population.

The SIT differs from classical biological control, which involves the introduction of exotic biological control agents, in the following key areas:

1. Sterile insects are not self-replicating and cannot become established in the environment.
2. Autocidal control is by definition intraspecific.
3. SIT used against an established pest never introduces an exotic species into the ecosystem where the SIT programme is being implemented.

The SIT has been used for nearly 50 years for eradication, suppression and control programmes of both plant and animal pests (e.g. Mediterranean fruit fly (medfly, *Ceratitis capitata*) and New World screwworm (NWS, *Cochliomyia hominivorax*). Because of the limited number of facilities for rearing and sterilization, sterile insects are often shipped for release in other locations. Transboundary shipments have gone from production facilities to release sites in countries throughout the world. Demand for SIT is rising and new commercial facilities may be constructed soon to meet this demand.

I-A Background on transboundary shipments

Transboundary shipments of sterile insects have been made on a continuous basis for the past 46 years. The first shipment of sterile NWS was from its production site at the USDA/APHIS mass rearing facility in Florida, USA, to the Caribbean island of Curaçao in 1954. This effort resulted in the eradication of the NWS from the island that same year. This was the first eradication of an insect pest population using the SIT.

Most of the transboundary shipments of sterile insects have originated from production facilities in North and Central America for shipment to at least 22 countries in 4 continents including the Americas, Europe, Africa and Asia (see Annex 3). One example is the ongoing shipment of sterile medfly pupae from the production factory in Tapachula, Chiapas, Mexico, to the packing and emerging facility in the southwest of Guatemala. Since 1979, biweekly ground and air shipments have been carried out amounting to 280 billion sterile flies (ca. 4,830 tons) in 21 years. Another important case is the ground and air shipment, since 1992, of 104 billion sterile NWS (ca. 1,733 tons) from the screwworm factory in Tuxtla Gutierrez, Chiapas, Mexico, to all of Central America, Panama and the Caribbean.

In Europe, most transboundary shipments of sterile insects have been carried out in support of SIT pilot projects. The first case involved sterile Mediterranean fruit flies shipped from the FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf, Austria, to the island of Procida, Italy, in 1970. There are some other examples of transboundary shipments of sterile insects produced in Europe such as the case of the 206 million sterile Mediterranean fruit flies shipped from the mass rearing facility in Madeira, Portugal to Israel during 1997/98.

Other cases involving Europe include transit shipments of sterile pupae from Guatemala, Central America, through Amsterdam, Frankfurt or Madrid, to Israel and South Africa and from Mexico, through Frankfurt, to Libya, (see Table in Annex 3).

In the past 46 years, at least 962 billion sterile insects (equivalent to about 18,000 tonnes) have been shipped domestically and internationally. None of these shipments has ever been prohibited from transit or entry for phytosanitary reasons by the 22 recipient countries or numerous transiting countries. The sterile insects are shipped by air cargo (commercial airlines or charter planes) or by ground in refrigerated trucks. They are packed in labelled, sealed containers to prevent contamination or escape. These safeguards are in place to protect the integrity of the sterile insects and not that of the public, property or the environment in the event of a massive escape. The same measures serve as safeguards against the hazards identified in this document, however, thereby greatly reducing any risk.

I-B Existing Guidelines

Internationally recognized guidelines on many steps in the mass rearing and sterilization of insects and quality control (materials used in production, the product and process) already exist (see References Section IX) but there are no internationally recognized guidelines for regulating shipment of sterile insects. Some countries do not regulate shipment of sterile insects, others only require labelling and documentation, and still others are regulating sterile insects under their biological control measures. In order to encourage a harmonized approach to national treatment of this method of plant pest control, some guidance on the risks involved will be very useful.

II. SCOPE

This discussion paper characterizes the risks involved with the transboundary shipment and importation (either in-transit through third countries or directly to the importing country) of sterile insects for use as autocidal control agents in control programmes of plant insect pests. Mass production site hazards and risks related to the release of sterile insects did not fall within the terms of reference of this Consultants Group.

Shipment of sterile, mass reared insects was considered including those developed through traditional selection and mutation breeding, for example sexing strains. Sterile insects resulting from strains which may be created artificially by genetic engineering or other modern biotechnology methods were excluded.

This discussion paper is also limited to the shipment of sterile insects resulting from radiation-induced sterility and does not deal with sterile insects resulting from the application of other sterilization techniques (e.g. chemosterilants or transgenically-induced sterilization).

III. HAZARD IDENTIFICATION

A key objective of the Consultants Group was to identify and characterize potential phytosanitary hazards associated with the transboundary shipment of sterile plant insect pests. The Consultants identified hazards and distinguished independent events leading to the occurrence of each hazard. This provided a format for estimating the likelihood and characterizing the consequences of each hazard in a scenario analysis[17]. Figure 1 shows the scenarios for each of the hazards.

Four potential hazards were identified as follows:

HAZARD	PRIMARY EVENT THAT COULD RESULT IN THIS HAZARD
1. Outbreak of target insect pest in a new area	Faulty sterilization
2. Increase of fitness of local pest population	Faulty sterilization
3. Unnecessary regulatory action initiated	Faulty ID of sterile insect
4. Introduction of exotic (new) contaminant organisms	Presence of hitch-hikers in shipments

The first two scenarios require failure of the sterilization treatment as the first event. This could mean absolute failure (i.e. the shipment was not treated) or that the treatment was less than necessary to meet the required specifications for sterility.

The second event that must occur in the first two scenarios is a breach of the package to allow for spillage or escape. It is assumed that in most situations this will be under adverse conditions (e.g. airport cargo handling environment). As a result, the pest must not only be liberated (event c), but it must also survive to escape into a favourable environment (event d). Finally, it must mate and reproduce for either hazard 1 or 2 to occur. However, in the case of hazard 2, the scenario recognizes that the introduction of new genetic material in itself does not present a risk unless an undesirable genetic trait is expressed and also has a selective advantage to become established in the population (event e).

The situation in hazard 3 is not related to biological consequences but rather based on regulatory actions (e.g. delimiting survey) that may be unnecessarily taken by the country where the pest is detected but not recognized as sterile. Adverse phytosanitary measures may be put in place by trading partners based on reporting the detection without distinguishing the pest as sterile.

Hazard 4, the introduction of exotic contaminating organisms, was not characterized in the same way as the other three hazards because it is a complex set of sub-scenarios depending on the nature of the contaminant organisms (e.g. parasitoids, virus, etc). This hazard is also different because it is not unique to sterile insects. Similar hazards exist with shipment of biological control agents and to some extent with any shipment. In fact, the sterile insect mass rearing process virtually eliminates any parasitoids.

In each of the three scenarios (hazards 1, 2 and 3) for which independent events were identified, the likelihood of each event occurring is represented by rough estimates of the probability (a point estimate). The product of the estimates for independent events in each scenario gives an overall estimate for the probability of the hazard occurring. It is noted that the mathematical relationship of these events means that where any event in a scenario is zero, the probability for the entire scenario is also zero.

The estimates are based on data, past programme records, and experience and expert opinion, primarily as regards fruit fly and some Lepidoptera species. They involve extremely rare events for which the primary source of evidence is the substantial history of experience with SIT shipments since 1954 and detailed knowledge of the technical/scientific aspects of the technology.

This approach was used to allow the comparison of risk levels between events and hazards associated with the transboundary shipment of sterile insects. It was not intended to be quantitatively precise, but more importantly to clarify the relative differences in magnitude. It is also useful to facilitate the comparison of phytosanitary risks associated with the transboundary shipment of sterile insects with those associated with other transboundary shipments (e.g. biological control agents).

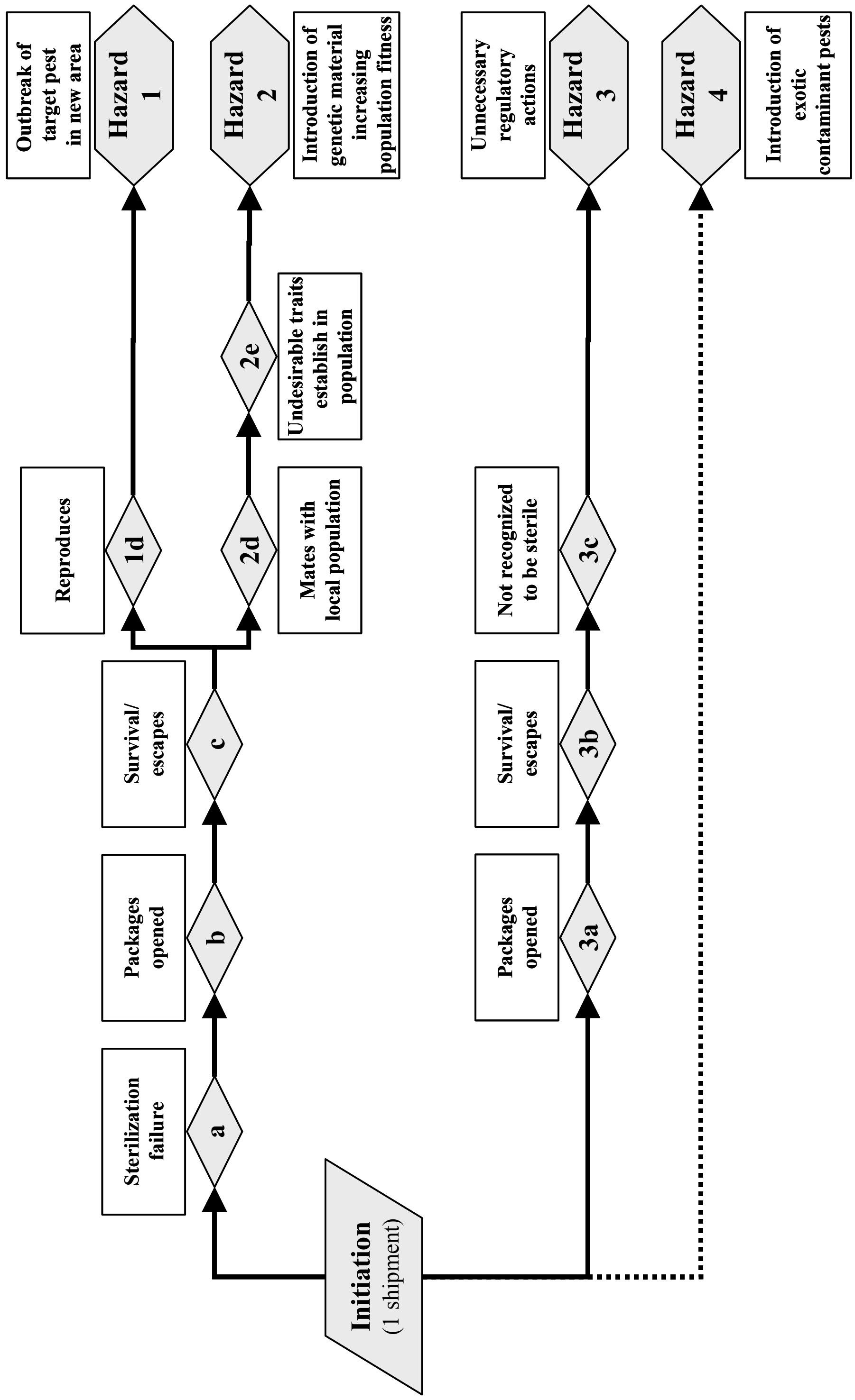
The scenario analysis process is limited to characterizing direct phytosanitary hazards associated with the range of insect plant pests historically and currently controlled by SIT for phytosanitary applications. It should be

[17] Reference for scenario analysis technique (L. Miller et. al., 1993).

noted that the scenarios are useful for pest risk management to the extent that they help to distinguish control points where risk-reducing measures may be applied.

The process does not consider indirect hazards or evaluate the risks against the benefits (e.g., increased pesticide use without SIT). In particular, it should be recognized that although the level of risk for any particular hazard may be the same for an importing and transit country, the transit country does not benefit to the same degree as the importing country from accepting this risk. In any case measures decided by either importing or transit countries should be technically justified (based on risk analysis or an international standard).

Figure 1. Hazard Scenarios for Transboundary Shipment of Sterile Insects



IV. LIKELIHOOD OF THE EVENT

IV-A Hazard 1: Outbreak of the target insect pest in a new area

Event *a*: Sterilization failure

An estimated 12,000 ground and air shipments of sterile insects have occurred since 1954 and two instances of partial failure to sterilize (1 confirmed and 1 unconfirmed) have been reported. The confirmed incident occurred in 1982 in a shipment of medflies from Costa Rica to Guatemala (S. Sanchez, personal communication, 1982) and the unconfirmed incident with a shipment of medflies from Peru to California, USA, in 1980 (Rohwer, 1987). Since then, international quality control standards were put in place and there have been no sterilization failures despite the significant increase in the use of SIT.

Current safeguards to prevent sterilization failure:

- Modern production facilities employ failsafe irradiation systems (i.e. physical and/or procedural) to prevent this.
- Each treated container has a dosimetry device that assures the container was irradiated.
- Minimum dosage received by all the insects far exceeds the dosage required to sterilize the females.
- Irradiators are equipped with automatic exposure settings that are tamper-proof.
- Procedures are observed for routine calibration of the equipment.
- Packages are clearly labelled as containing irradiated insects.
- A sample of insects from each shipment is bio assayed for sterility at factory and release site for quality control.

*The likelihood was estimated by the consultants group to be an **extremely rare event** with an estimated probability of* $\mathbf{0.5 \times 10^{-6}}$

Event *b*: Packages open

In addition to the above event, it would be unlikely for the packages carrying the fertile insects to open because:

- From tens of thousands of containers shipped since 1954 there has been no documented case of breakage of shipping package.
- Using one of the longest routes (i.e. Guatemala City-Miami-Frankfurt-Tel Aviv) from 1998 to 2001, 1 out of over 400 shipments was never recovered. In this event, due to the length of time involved, highly perishable material (i.e. sterile insects) would not survive.
- Current safeguards to prevent mishandling leading to breakage of package include:
  - All consignments are double packaged, some triple packed, and then sealed.
  - Consignments are closely tracked with commercial motivation for rapid transit of highly perishable material.
  - Rapid feedback from receiver when the package is delayed.
  - Size and weight of package designed to minimize breakage.
  - All packages are appropriately labelled (e.g. fragile, biological material) and numbered.
- Content of package does not attract theft.

*The likelihood was estimated by the consultant group to be an **extremely rare event** with an estimated probability of* 1×10^{-5}

Event *c*: Survives/escapes

In addition to the above events, the fertile insects would be unlikely to survive and disperse to a favourable habitat because:

- Immediate in-transit area is inhospitable (i.e. lack of water, food, wrong temperature, no host, concrete/asphalt substrate). Presence of insecticide/toxicants at airports.
- Airport security prevents unauthorized removal of packages from the airport.
- Limited survival from pupal to adult stage, and even lower chance to survive to sexual maturity and disperse because of high predation, desiccation, starvation, drowning, temperature stress, etc.

*The likelihood was estimated by the consultant group to be a **fairly unlikely event** with an estimated probability of* 1×10^{-3}

Event *d*: Reproduces

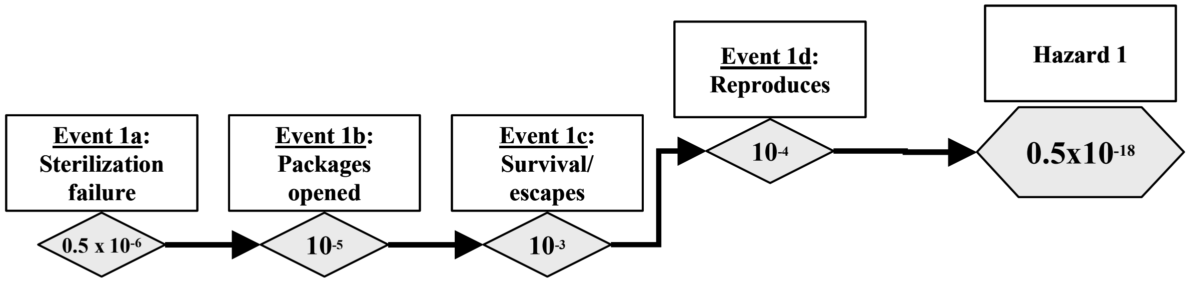
In addition to the above events, reproduction by the escaped insects would be unlikely because:

- Event may occur during seasonally inhospitable period.
- Climatic factors not suitable for establishment.
- Factory strain has lower fitness for survival in nature.
- Too few survivors to disperse and find suitable environment, mating partners and hosts.

The likelihood was estimated by the consultant group to be a ***rare event*** *with an estimated probability of* $\mathbf{1 \times 10^{-4}}$

For the scenario for hazard 1 the likelihood of all four events occurring was estimated as a negligible risk with a probability of $\mathbf{0.5 \times 10^{-18}}$

Summary of hazard 1: Outbreak of the target insect pest in a new area



IV-B Hazard 2: Increase of fitness of the local pest population through introduction of genetic material from the escaped insects

For this scenario to take place, events 2a, 2b and 2c must occur. These have the same values as 1a, 1b and 1c. In addition, events d and e must occur:

Event d: Escaped insects reach sexual maturity and mate with local population

In addition to the above events, the escaped insects would be unlikely to reach maturity and mate. This event is very similar to *1d* but assumes that an established pest population exists in the area and that wild mates are receptive to mating.

The likelihood was estimated by the consultants group to be a ***fairly unlikely event*** *with an estimated probability of* $\mathbf{1 \times 10^{-3}}$.

Event e: Undesirable traits established in the population

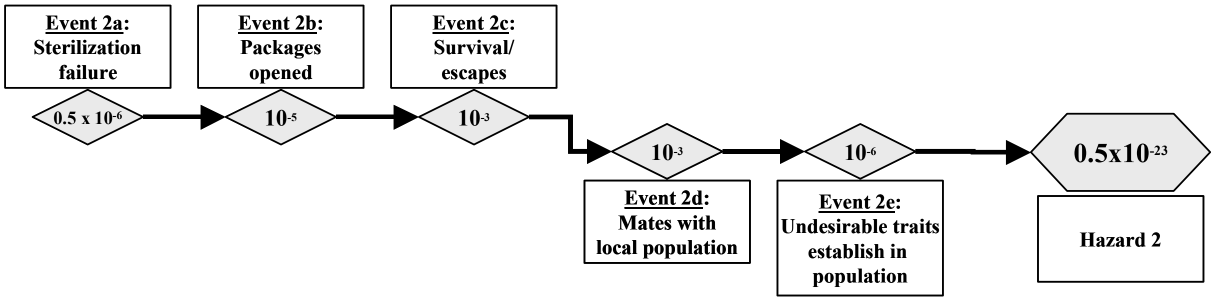
In addition to the above events, the escaped insects would have to possess traits that convey a selective advantage leading to increased fitness. Furthermore, these traits would have to become established in the population. However, this is extremely unlikely because:

- Most introductions of genetic material have neutral or even a detrimental effect on the population. Furthermore, because of the small numbers of escaped insects, it is unlikely that these traits would become established in the wild population.
- Under mass rearing conditions over many generations, all laboratory strains are known to loose their fitness to survive under natural conditions, therefore they are highly unlikely to carry genetic traits that would increase the fitness of the wild population.
- In addition, the only known traits that have been introduced into mass reared strains through traditional selection and mutation breeding (i.e. markers and sexing features) are detrimental (e.g. temperature sensitive lethal).

The likelihood was estimated by the consultants group to be an ***extremely rare event*** *with an estimated probability of* $\mathbf{1 \times 10^{-6}}$.

For scenario 2 the likelihood of all five events occurring was estimated as a negligible risk of $\mathbf{0.5 \times 10^{-23}}$

Summary of hazard 2: **Increase of fitness of the local pest population through introduction of genetic material from the escaped insects.**



IV-C Hazard 3: Unnecessary regulatory actions initiated due to failure to recognize the detected insect as sterile

Event 3a (i.e. packages opened) is identical to event 1b. Event 3b (i.e. survives and escapes) is the same as event 1c.

Event c: Not recognized to be sterile

In addition to the above events, the escaped insects would have to be detected and not recognized as sterile.

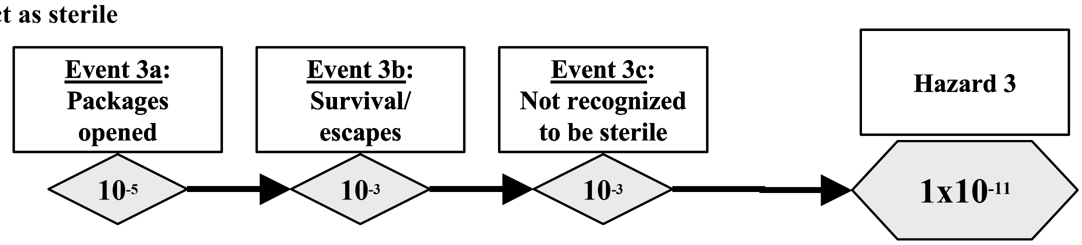
For this to occur the insect must be of regulatory significance:

- The plant protection authorities would have to be conducting detection surveys.
- The plant protection authorities would have to fail to recognize that this could be a sterile insect, which is an unlikely event. Those countries that are most likely to take a regulatory action have standard operation procedures that recognize the possibility of capturing sterile insects.
- The sterile insect marking process and cytological identification for sterility would have to fail.

The likelihood was estimated by the consultant group to be a ***fairly unlikely event*** *with an estimated probability of* ***1 x*** 10^{-3}.

For scenario 3 the likelihood of all three events occurring was estimated as a negligible risk of 1 x 10^{-11}.

Summary of hazard 3: Unnecessary regulatory actions initiated due to failure to recognize the detected insect as sterile



IV-D Hazard 4: Introduction of exotic (new) contaminant organisms

The introduction of exotic contaminant organisms was characterized in a different way because of the complexity of the sub-scenarios involved depending on the nature of the contaminant organisms (e.g. parasitoids versus micro-organisms). This hazard is also different because it is not unique to sterile insects. Similar hazards exist with shipment of biological control agents and to some extent with any shipment. Therefore it was compared to the risks from the shipment of biological control agents, which is widely practiced.

The risk of sterile insect shipments introducing exotic organisms were estimated to be considerably smaller based on the following considerations:

- There is no documented evidence that such an event has occurred during the past 46 years of sterile insect shipping.
- The items being shipped undergo sterilization. This would effectively reduce the risk of introducing unwanted parasitoids.
- Wild-collected organisms are never shipped for SIT purposes. The product is mass reared over many generations under quality control procedures aimed at eliminating unwanted organisms.

- The standard operating procedures for insect mass rearing specifically provide mechanisms to prevent unwanted organisms.
- Biological control agents are sometimes shipped with live hosts or prey. Sterile insects are not.

For scenario 4, the consultants estimated that this risk would be many-fold less likely than the risk of introducing exotic organisms involved when moving biological control agents.

V. CONSEQUENCES IN CASE THE IDENTIFIED HAZARDS OCCURRED

Assuming that the identified hazards have occurred, the expert group described the following potential consequences:

Hazard 1: Outbreak of the target insect pest in a new area

The consequence of this hazard is the incursion or establishment of a serious insect plant pest. Negative impact of the new pest could include:

- Decrease in production of crops.
- Reduction in quality.
- Increase in production costs.
- Impact on trade.
- Impact on the environment.

These consequences apply to both incursions and establishment. In the case of incursions, the negative impact would be limited in scope and duration. This is because for an incursion, the conditions would not be suitable for permanent pest establishment (e.g. pest not able to survive winter or summer temperatures). However, in the event of pest establishment, eradication would be an option since SIT and other eradication tools are available for the species that are currently shipped as sterile insects.

Hazard 2: Increase of fitness of the local pest population through introduction of genetic material from the escaped insects.

The consequences of the existing local pest population could increase as a result of the introduction of new genetic material. This negative impact could be:

- Decreased production on already affected crops.
- Increased cost on already affected crops.
- Losses on other crop species.
- Environmental impact.
- Impact on trade.

With the existence of a local population, however, control practices may already be in place that will effectively manage the fitter pest. This may reduce the consequences.

Hazard 3: Unnecessary regulatory actions initiated due to failure to recognize the detected insect as sterile

This would apply only to pests subjected to an active surveillance programme. The detection and failure to recognize the insect as sterile could trigger several different actions:

- An increase in trapping (i.e. delimiting trapping) to assess the status of the detection.
- The initiation of an emergency programme for eradication.
- Disruption of internal movement and marketing by domestic regulatory actions.
- Prohibition of host product by a trading partner.

The implementation of these actions could have significant short-term financial implications.

Hazard 4: Introduction of exotic (new) contaminant organisms

The introduction of an exotic organism into a new ecosystem can have the following negative impacts:

- Direct damage on agricultural crops if the introduced organism is an exotic plant pest.
- Indirect damage on agricultural crops if introduced organism has a negative impact on beneficial organisms (pollinators, predators and parasites).
- Change in biodiversity and natural ecosystem.

This hazard is not unique to the shipment of sterile insects, and therefore should be considered in comparison to or in the context of the same hazard associated with shipments of other commodities, including non-biological shipments.

VI. ASSESSED RISK

Risk is the product of the likelihood of the hazard times the consequences. The potential consequences from the identified hazards could be significant. However, the extremely low likelihood of the hazards occurring indicates an overall negligible risk.

VII. CONCLUSIONS

The Consultants held detailed discussions and reviewed reference documents taking into consideration the scientific, technical and operational aspects of the Sterile Insect Technique (SIT) as applied to plant protection. Potential biological hazards and associated risks were identified for transboundary shipment of sterile insects for use in SIT programmes.

The consultants concluded the following:

A. Evidence indicates that SIT is likely to become more widely used. There is also a shift from government to private responsibility for certain aspects of the technology. This will require a more formal approach to activities involving more than one country. This is particularly relevant to production that results in transboundary shipments of the sterile insects.

B. The SIT has been used for nearly 50 years against insect pests of plants and animals. During this time, standard operating procedures have been developed by most individual programmes. In some cases, international standards have been developed and are in use worldwide. For fruit fly species, the most important of these are the quality control and dosimetry manuals[1] (FAO/IAEA/USDA, 1998 and FAO/IAEA, 2000). The proper application of these manuals precludes the hazards identified by the Consultants Group from occurring.

C. There is a need for an internationally accepted code of conduct (or similar document) relating to transboundary shipments of sterile insects for use in SIT programmes. The International Plant Protection Convention (IPPC) is the international standard setting body for phytosanitary measures. Since the SIT is also used against insect pests of veterinary and medical importance, livestock insect pests and insect vectors of medical importance should be considered by the appropriate bodies in the near future.

D. The Consultants Group identified the hazards and assessed the risks associated with the transboundary shipment of sterile insects for SIT programmes. Both the likelihood and the consequences were considered for each of the hazards identified. A series of sequential events would be required for any of these potential hazards to occur. None of the events alone would constitute a hazard (refer to Figure 1).

E. The hazards identified, potential consequences and likelihood of the hazards occurring were:

1. Failure of sterilization, either total or partial, resulting in the target insect becoming an established pest in a new area, with the likelihood of 0.5×10^{-18}.
2. Introduction of new (intra-specific) genetic material into an established pest population by the "sterile insects", resulting in a more damaging insect pest, with the likelihood of 0.5×10^{-23}.
3. Failure to recognize a detected insect as sterile, resulting in an unnecessary and perhaps costly regulatory action, with the likelihood of 1×10^{-11}.
4. Introduction of an exotic contaminant organism, resulting in a new pest becoming established, was estimated to involve many folds less risk than from the movement of biological control agents, a risk already widely accepted.

F. Because of the sequence of events required for any of the above hazards to occur, the Consultants Group concluded that transboundary shipment would result in negligible risk with the use of FAO/IAEA operating procedures[18] regarding sterilization, handling/packaging and shipment of sterile insects.

[18] Comprehensive FAO/IAEA standard operating procedures exist for fruit fly species. For other plant pest species controlled by SIT, best practices are in place and standard procedures will be harmonized internationally over time. The Consultants Group believes that the risk will be negligible from transboundary shipment of these other species as well, when best practices are applied.

VIII. RECOMMENDATIONS

The Consultants Group recommends that this discussion paper be sent to the IPPC Secretariat for consideration by the ICPM as the basis for a standard. The Group also recommend that this standard be separate from the International Standard for Phytosanitary Measures number 3 on biological control agents.

Furthermore, the consultants recommend that the appropriate international bodies should assess the risks from transboundary shipment of insect pests of livestock and insects of medical importance controlled through SIT, and develop harmonized guidance.

IX. REFERENCES

Relevant guidelines for SIT

American Society for Testing and Materials (ASTM) (1999), Standard Guide for Irradiation of Insects for Sterile Release Programs. Designation: ASTM E 1940 - 98. 11 pp.

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IPPC (1998a) Guidelines for surveillance. ISPM Pub. No. 6, FAO, Rome.

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ANNEX 2. ACRONYMS, TERMS AND DEFINITIONS

ACRONYMS

APHIS	Animal and Plant Health Inspection Service of the USDA
FAO	Food and Agriculture Organization
IAEA	International Atomic Energy Agency
ICPM	Interim Commission on Phytosanitary Measures (member countries of the IPPC)
IPPC	International Plant Protection Convention
ISPM	International Standard on Phytosanitary Measures (established under the IPPC)
NWS	New World Screwworm
OIE	Office International des Epizooties
QC	Quality Control
SIT	Sterile Insect Technique
USDA	United States Department of Agriculture
WHO	World Health Organization

TERMS AND DEFINITIONS

Area* – An officially defined country, part of a country, or all or parts of several countries.

Certificate* – An official document which attests to the phytosanitary status of any consignment affected by phytosanitary regulations.

Classical biological control* – The intentional introduction and permanent establishment of an exotic biological agent for long-term pest control.

Consignment* – A quantity of plants, plant products and/or other articles being moved from one country to another and covered, when required, by a single phytosanitary certificate (a consignment may be composed of one or more lots).

Consignment in transit* – Consignment which is not imported into a country but passes through it to another country, subject to official procedures which ensure that it remains enclosed, and is not split up, not combined with other consignments nor has its packaging changed. (draft revised definition)

Control (of a pest)* – Suppression, containment or eradication of a pest population.

Detection survey* – Survey conducted in an area to determine if pests are present.

Entry (of a pest)* – Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled.

Entry (of a consignment)* – Movement through a point of entry into an area.

Eradication* – Application of phytosanitary measures to eliminate a pest from an area.

Establishment* – Perpetuation, for the foreseeable future, of a pest within an area after entry.

Exotic* – Not native to a particular country, ecosystem or eco-area (applied to organisms intentionally or accidentally introduced as a result of human activity). As this Code is directed at the introduction of biological control agents from one country to another, the term "exotic" is used for organisms not native to a country.

Hazard – Elements or events which represent potential harm; an adverse event or adverse outcome (based on OIE definition).

Hazard (phytosanitary) – Injury or deleterious effects caused by pests to plants, plant products or the health of plants in an ecosystem.

Incursion – Presence of a pest population within an area where it is capable of causing economic damage but not capable of establishment. (based on Art VII.3 of IPPC text)

Occurrence* – The presence in an area of a pest officially recognized to be indigenous or introduced and/or not officially reported to have been eradicated. (draft revised definition)

Outbreak* – An isolated, recently detected pest population. (draft revised definition)

Parasite* – An organism which lives on or in a larger organism, feeding upon it.

Parasitoid* – An insect parasitic only in its immature stages, killing its host in the process of its development, and free living as an adult.

Pathogen* – Micro-organism causing disease.

Pest* – Any species, strain or biotype of plant, animal or pathogenic agent injurious to plant or plant products.

Pest risk analysis* – the process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it.

Pest risk assessment* – Evaluation of the probability of the introduction and spread of a pest and of the associated potential economic consequences.

Pest risk management* – Evaluation and selection of management options to reduce the risk of introduction and spread of a pest.

Pest status (in an area)* – Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information.

Phytosanitary action* – An official operation, such as inspection, testing, surveillance or treatment, undertaken to implement phytosanitary regulations or procedures.

Phytosanitary certificate* – Certificate patterned after the model certificates of the IPPC.

Phytosanitary measure* – Any legislation, regulation, or official procedure having the purpose to prevent the introduction and/or spread of pests.

Phytosanitary procedure* – Any officially prescribed method for implementing phytosanitary regulations including the performance of inspections, tests, surveillance or treatments in connection with regulated pests.

Point of entry* – Airport, seaport or land border point officially designated for the importation of consignments, and/or entrance of passengers.

Release (into the environment)* – Intentional liberation of an organism into the environment (see also introduction and establishment).

Release (of a consignment)* – Authorization for entry after clearance.

Sterility (radiation induced) – A condition in which sperm or eggs from irradiated reproducing individuals do not result in fertile offspring following fertilization.

Suppression* – The application of phytosanitary measures in an infested area to reduce pest populations.

Surveillance* – an official process which collects and records data on pest occurrence of absence by survey, monitoring or other procedures.

Survey* – An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area.

Treatment* – Officially authorized procedure for the killing, removal or rendering infertile of pests.